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

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

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

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PROOF

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3 **1 Skeletal muscle IL-15/IL-15R $\alpha$  and myofibrillar protein synthesis after resistance**  
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5 **2 exercise**  
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9 4 Alberto Pérez-López<sup>1,2,3,4</sup>, James McKendry<sup>3,5</sup>, Marcos Martin-Rincon<sup>4,6</sup>, David Morales-  
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51 30  
52 31 **Running head:** IL-15/IL-15R $\alpha$  in Resistance Exercise  
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## 32 ABSTRACT

33 *In vitro* and *in vivo* studies described the myokine IL-15 and its receptor IL-15R $\alpha$  as  
34 anabolic/anti-atrophy agents, however the protein expression of IL-15R $\alpha$  has not been measured  
35 in human skeletal muscle and data regarding IL-15 expression remain inconclusive. The  
36 purpose of the study was to determine serum and skeletal muscle IL-15 and IL-15R $\alpha$  responses  
37 to resistance exercise session and to analyse their association with myofibrillar protein synthesis  
38 (MPS). Fourteen participants performed a bilateral leg resistance exercise composed of 4 sets of  
39 leg press and 4 sets of knee extension at 75% 1RM to task failure. Muscle biopsies were  
40 obtained at rest, 0, 4 and 24h post-exercise and blood samples at rest, mid-exercise, 0, 0.3, 1, 2,  
41 4 and 24h post-exercise. Serum IL-15 was increased by ~5.3-fold immediately post-exercise,  
42 while serum IL-15R $\alpha$  decreased ~75% over 1h post-exercise ( $P<0.001$ ). Skeletal muscle IL-  
43 15R $\alpha$  mRNA and protein expression were increased at 4h post-exercise by ~2-fold ( $P<0.001$ )  
44 and ~1.3-fold above rest ( $P=0.020$ ), respectively. At 24h post-exercise IL-15 ( $P=0.003$ ) and IL-  
45 15R $\alpha$  mRNAs increased by ~2-fold ( $P=0.002$ ). Myofibrillar fractional synthetic rate between 0-  
46 4h was associated with IL-15R $\alpha$  mRNA at rest ( $r=0.662$ ,  $P=0.019$ ), 4h ( $r=0.612$ ,  $P=0.029$ ) and  
47 24h post-exercise ( $r=0.627$ ,  $P=0.029$ ). Finally, the muscle IL-15R $\alpha$  protein up-regulation was  
48 related to Leg press 1RM ( $r=0.688$ ,  $P=0.003$ ) and total weight lifted ( $r=0.628$ ,  $P=0.009$ ). In  
49 conclusion, IL-15/IL-15R $\alpha$  signalling pathway is activated in skeletal muscle in response to a  
50 session of resistance exercise.

51 **Keywords:** Myokines, IL-15/IL-15R $\alpha$  axis, strength training, muscle protein  
52 synthesis/breakdown.

## 53 INTRODUCTION

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

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

70 Although part of the effects of IL-15 are mediated by its binding to IL-15R $\alpha$  (Dubois et  
71 al., 2002; Duitman et al., 2008; Sato et al., 2007), this alpha-receptor may also exert functions  
72 independent from IL-15 in skeletal muscle. IL-15R $\alpha$  may have a role in determining the  
73 phenotype and fatigability of muscle fibers, and mitochondrial fuel utilization (Loro et al.,  
74 2015; O'Connell et al., 2015; O'Connell et al., 2015). In addition, human studies indicate that  
75 IL-15R $\alpha$  may be involved in muscle hypertrophy and strength gains after resistance training  
76 (Pistilli et al., 2008; Riechman et al., 2004). In this regard, two single nucleotide polymorphism  
77 (SNPs) in exon 7 and 4 of the IL-15R $\alpha$  could explain part of the variability in the hypertrophy  
78 observed after 10 weeks of whole-body resistance training (Riechman et al., 2004), whereas IL-  
79 15R $\alpha$  SNPs, rs2296135 and rs22228059, were positively associated with pre- and post-exercise

1 80 isometric strength and muscle volume, respectively, after 12 weeks of resistance training of the  
2  
3 81 flexor-extensor muscles of the elbow (Pistilli et al., 2008).  
4

5 82 Despite the potential implication of IL-15 and IL-15R $\alpha$  in skeletal muscle  
6  
7 83 anabolic/catabolic balance, direct evidence is lacking as no human study has determined  
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9 84 whether changes in skeletal muscle IL-15R $\alpha$  mRNA and protein expression are associated with  
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11 85 protein synthesis. Recently, we reported a ~2-fold elevated myofibrillar protein synthesis  
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13 86 (MPS) response during the first four hours (0-4h) after a single session of resistance exercise in  
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15 87 healthy young males (McKendry et al., 2016), however, skeletal muscle IL-15 and IL-15R $\alpha$   
16  
17 88 were not determined. Therefore, the aim of this study was to determine whether circulating and  
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19 89 skeletal muscle IL-15 and IL-15R $\alpha$  might have a role in the regulation of myofibrillar protein  
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21 90 synthesis, after a single session of resistance exercise.  
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25 91 We hypothesised that skeletal muscle IL-15 and IL-15R $\alpha$  expressions would be up-  
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27 92 regulated after a single session of resistance exercise and that IL-15 and IL-15R $\alpha$  expression in  
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29 93 skeletal muscle would be associated with myofibrillar protein synthesis.  
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## 33 95 **MATERIALS AND METHODS**

### 34 96 **Participants**

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36 97 A full description of the methods, study design and participant characteristics, from which part  
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38 98 of current data are drawn, has been previously published (McKendry et al., 2016). Volunteers  
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40 99 were aged from 18 to 35 years and had been participating in resistance training programmes > 2  
41  
42 100 days/week during  $\geq$  1 year prior to start of current study. Subjects' characteristics are presented  
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44 101 in Table 1. Prior to study enrolment all procedures were explained to participants who then  
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46 102 gave their written informed consent. Ethical approval was obtained through the NHS Black  
47  
48 103 Country Research Ethics Committee (13/WM/0455) in accordance with the latest version (7<sup>th</sup>)  
49  
50 104 of the Declaration of Helsinki.  
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## 106 **Experimental design**

107 All participants reported to the School of Sport, Exercise and Rehabilitation Sciences  
108 (SportExR) laboratory on 3 separate occasions. During visit one, participants underwent  
109 preliminary assessments of body composition and maximal leg strength. Then, within a period  
110 of 8 days, the volunteers returned to the laboratory for the experimental trial, which consisted of  
111 a single session of bilateral lower-limb resistance exercise with muscle biopsies obtained at  
112 baseline, immediately after (0h), 4 and 24h post-exercise, and blood samples at baseline, mid-  
113 exercise, and 0, 0.3, 1, 2, 4 and 24h post-exercise to assess the systemic and skeletal muscle  
114 responses of the IL-15/IL-15R $\alpha$  axis. As part of the original investigation (McKendry et al.,  
115 2016), participants were matched in pairs based on anthropometric, strength and training  
116 characteristics before to be randomly allocated to either 1-min or 5-min of passive rest between  
117 resistance exercise sets. Since no significant differences were observed in circulating or  
118 intramuscular IL-15 and IL-15R $\alpha$  measurements between the 1-min (N = 7) and 5-min groups  
119 (N = 7), participants were treated as a single group for the purpose of the present analyses. Of  
120 the 16 participants included in the original study, 14 were analysed in the present investigation  
121 due to insufficient muscle tissue in the two subjects excluded.

122

## 123 **Experimental protocol**

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



1 133 under local anaesthesia (1% lidocaine). Skeletal muscle sample was cleaned from any fat or  
2  
3 134 connective tissue before being frozen in liquid nitrogen. Following the muscle biopsy,  
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5 135 participants completed a session of bilateral lower-extremity resistance exercise on leg press  
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7 136 and knee extension machines. Exercise consisted of four sets of 8-15 repetitions per exercise at  
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9 137 75% of 1RM, each set performed to task failure. At the end of the last repetition, a second  
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11 138 muscle biopsy was obtained ~3 cm proximal from the first biopsy through a new incision.  
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13 139 Immediately after the second biopsy, the volunteers ingested 25 g of whey protein isolate  
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15 140 (MyProtein, Cheshire, UK) dissolved in 400 mL of water. During the next four hours,  
16  
17 141 participants rested supine and then a third muscle biopsy was obtained from a new incision, ~3  
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19 142 cm proximal to the second biopsy. The following morning at 7.00 h, participants returned to the  
20  
21 143 laboratory after a 10-12h overnight fast and a cannula was inserted into a forearm vein to obtain  
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23 144 a blood sample followed by the fourth and last biopsy, which was obtained from the *vastus*  
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25 145 *lateralis* of the contralateral leg.  
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147 The participants received three standardised meals for consumption the evening prior to  
32  
33 148 the experimental trial, as well as the afternoon and evening after the experimental trial. The diet  
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35 149 was composed by ~97 g of CHO (~58%), ~34 g of protein (~20%) and ~37 g of fat (~22%)  
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37 150 with an energy content of ~871 kcal per meal. Consumption of ethanol or caffeine was not  
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39 151 allowed 24h before the experiments neither during the study.  
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## 153 **Blood Analysis**

### 154 ***Serum IL-15 and IL-15R $\alpha$***

155 After collection, all blood samples were centrifuged for 15 minutes at 1000 g, aliquoted and  
50  
51 156 stored at -80 °C. Two high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits were  
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53 157 used to determine the serum concentration of IL-15 and IL-15R $\alpha$  in duplicates. IL-15 was  
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55 158 measured using Human IL-15 Quantikine ELISA kit (R&D Systems, MN, USA) recognizing  
56  
57 159 both natural and recombinant human IL-15 (range: 0.49 – 62.5 pg/mL; intra- and inter-assay

1 160 coefficients of variation (CV) were 4.2 and 7.4%, respectively). Serum IL-15R $\alpha$  was measured  
2  
3 161 using Human IL-15 receptor subunit alpha ELISA kit (Wuhan EIAab Science, Wuhan, China)  
4  
5 162 recognizing both natural and recombinant human IL-15R $\alpha$  (range: 0.49 – 62.5 pg/mL; intra-  
6  
7 163 and inter-assay CVs were 4.4 and 7.8%, respectively).  
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10 164

## 11 165 **Muscle Tissue Analysis**

### 12 166 *Protein extraction and Western Blot procedures.*

13  
14 167 Approximately 25-30 mg of muscle tissue was powdered on dry ice using a Cellcrusher<sup>TM</sup> tissue  
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16 168 pulveriser (Cellcrusher limited, Cork, Ireland) and a sucrose lysis buffer was used to prepare the  
17  
18 169 samples for Western Blot as previously described (Philp et al., 2011). Equal amounts of protein  
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20 170 (35  $\mu$ g per sample) were boiled for 5 min in 1 x Laemmli sample buffer, separated on 10%  
21  
22 171 SDS-PAGE gels (Bio-Rad, Copenhagen, Denmark) for 45 min and transferred to  
23  
24 172 polyvinylidene difluoride (PVDF) membranes at constant voltage and 0.4 A for 1.5 h.  
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26 173 Subsequently, membranes were incubated overnight with primary antibodies against IL-15 (sc-  
27  
28 174 7889) and IL-15R $\alpha$  (sc-271366), purchased from Santa Cruz Biotechnology (Dallas, USA).  
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30 175 Both antibodies were diluted into BSA-blocking buffer containing 4% bovine serum albumin in  
31  
32 176 Tris-buffered saline with 0.1% Tween 20. Antibody specific labelling was revealed by  
33  
34 177 incubation with an HRP-conjugated goat anti-rabbit (IL-15) or anti-mouse (IL-15R $\alpha$ ) antibodies  
35  
36 178 (1:5000), both diluted in 5% blotto blocking buffer and visualised with ECL Western blotting  
37  
38 179 detection system using a ChemiDoc XRS (Bio-Rad, Copenhagen, Denmark). Imaging and band  
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40 180 quantification were performed using the Quantity One 1-D Analysis software (Bio-Rad,  
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42 181 Copenhagen, Denmark). Test samples were run together with a control sample from a subject  
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44 182 who did not take part in the study. The control sample was loaded in three different lanes and  
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46 183 used as an internal control for inter-gel variability. Overall, the mean CVs of the controls were  
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48 184 13.9% (IL-15) and 12.3% (IL-15R $\alpha$ ). Control samples and a total protein staining-technique  
49  
50 185 method (reactive brown) were used to accurate protein quantification for loading control.  
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1 187 ***RNA Isolation and quantitative real-time reverse transcription polymerase chain reaction***  
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3 188 ***(qRT-PCR).***

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5 189 Approximately 15-20 mg of skeletal muscle tissue was used for the RNA isolation. The RNA  
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7 190 was extracted by guanine-phenol-chloroform isothiocyanate procedures using TRIzol  
8  
9 191 (Invitrogen, Carlsbad, CA, USA). Then, RNA was recovered from the aqueous phase by  
10  
11 192 precipitation; the amount and purity was measured by optical density at 260/280 nm and  
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13 193 260/230 nm in a NanoDrop ND-100 spectrophotometer (Thermo Fisher Scientific Inc., DE,  
14  
15 194 USA).

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18 195 Reverse transcription was performed to synthesize cDNA from 200 ng of the total RNA  
19  
20 196 using Oligo dT primers (GE Healthcare Bio-Sicences, Buckinghamshire, UK) and M-MLV  
21  
22 197 reverse transcriptase enzyme (Invitrogen, Carlsbad, CA, USA). The cDNA was amplified using  
23  
24 198 the primers presented in Table 2 (self-designed and tested in skeletal muscle from human  
25  
26 199 donors, data not shown). The qRT-PCR mixture was composed by 5  $\mu$ L of the inverse  
27  
28 200 transcription product (cDNA) diluted 1:20, 10  $\mu$ L of iQ SYBR Green Supermix (Bio-Rad,  
29  
30 201 Copenhagen, Denmark) and 1  $\mu$ L (6 mM) of the primer selected. The final reaction volume (20  
31  
32 202  $\mu$ L) was used to perform the qRT-PCR in a StepOnePlus Real-Time PCR System (Applied  
33  
34 203 Biosystems, Foster City, CA, USA). All samples were subjected to an initial stage of 10 min at  
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36 204 95°C. The conditions for PCR amplification were as follows: 45 cycles of 95 °C for 15 s, 60 °C  
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38 205 for 30 s and 72 °C for 1 minute, for both IL-15 and IL-15R $\alpha$ . Finally, mRNA expressions of IL-  
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40 206 15 and IL-15R $\alpha$  were determined in triplicates, and normalized using  $\beta$ -actin and  
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42 207 glyceraldehyde 3-phosphate-dehydrogenase (GAPDH) as housekeeping genes.  $\beta$ -actin and  
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44 208 GAPDH expression remained unchanged.

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51 210 ***Myofibrillar and plasma tracer enrichment.***

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53 211 Procedures for muscle myofibrillar protein isolation, plasma-free amino acid extraction and  
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55 212  $^{13}\text{C}_6$  phenylalanine enrichment, and calculation of myofibrillar fractional synthetic rate (FSR) at  
56  
57 213 rest, 0-4h and 24-28h post-exercise are described in McKendry et al. (2016).

1  
2 214 Briefly, a primed continuous infusion of L-[ring- $^{13}\text{C}_6$ ]phenylalanine (prime,  $2 \mu\text{mol kg}^{-1}$ ;  
3  
4 215 infusion,  $0.05 \mu\text{mol kg}^{-1} \text{min}^{-1}$ ; Cambridge Isotope Laboratories, Andover, MA, USA) was  
5  
6 216 implemented during both experimental trial days in conjunction with muscle biopsy and blood  
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8 217 sampling. In both experimental days, the infusion was initiated immediately after the drawn of  
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10 218 the first blood sample ( $\sim 7.05$  h) and finished when the last muscle biopsy sample of the day was  
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12 219 obtained ( $7.5$  h and  $5.5$  h after the beginning of the infusion on day 1 and 2, respectively). Upon  
13  
14 220 thawing, plasma samples were purified on cation-exchange columns. The amino acids were  
15  
16 221 then converted to their N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA)  
17  
18 222 derivative. Plasma [ $^{13}\text{C}_6$ ]phenylalanine enrichment was determined by gas chromatography–  
19  
20 223 mass spectrometry (GCMS; model 5973; Hewlett Packard, Palo Alto, CA, USA) by monitoring  
21  
22 224 ions 234/240. The myofibrillar protein fraction was extracted and hydrolysed overnight.  
23  
24  
25 225 Constituent amino acids in the myofibrillar fraction were purified on cation-exchange columns.  
26  
27 226 Amino acids in the myofibrillar fraction were then converted to their N-acetyl-n-propyl ester  
28  
29 227 derivative. Plasma [ $^{13}\text{C}_6$ ]phenylalanine enrichment was determined by gas chromatography–  
30  
31 228 mass spectrometry (GC-C-IRMS; Delta-plus XP; Thermofinnigan, Hemel Hempstead, UK) by  
32  
33 229 monitoring ions 44/45. Pre-infusion and mean plasma [ $^{13}\text{C}_6$ ]phenylalanine enrichment were  
34  
35 230 used as a proxy for basal muscle protein enrichment and to determine an “estimated”  
36  
37 231 intracellular precursor enrichment, respectively. The fractional synthesis rate (FSR) of the  
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39 232 myofibrillar protein fraction was calculated from the incorporation of [ $^{13}\text{C}_6$ ]phenylalanine into  
40  
41 233 protein using the standard precursor-product model (Wolfe & Chinkes, 2005).  
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44 234

### 46 235 **Statistical Analysis**

47  
48 236 Data collected in the study were analysed using the statistical package SPSS v. 22.0 (SPSS Inc.,  
49  
50 237 Chicago, IL, USA), and Graph Prism 6 (GraphPad software, Inc. La Jolla, CA, USA). Firstly, a  
51  
52 238 Shapiro-Wilks was used to test the normality of the data ( $P > 0.05$ ). Subsequently, non-  
53  
54 239 normally distributed variables were logarithmically transformed. Circulating and skeletal  
55  
56 240 muscle expression of IL-15 and IL-15R $\alpha$  were analysed between groups (1- vs. 5-min rest)  
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241 using a two-way, repeated measures ANOVA (time x condition). The area under the curve  
242 (AUC) was determined using trapezoid method and compared between groups using a paired  
243 Student's t-test. Since no significant differences were observed between the 1- and 5-min  
244 recovery, both groups were combined for further analyses.

245 To determine time effects of the intervention on serum, protein and mRNA levels of IL-  
246 15 and IL-15R $\alpha$ , ANOVA for repeated measures was performed. Tukey HSD correction was  
247 used as *post-hoc* test when significant differences were detected. Finally, linear regression  
248 analysis was carried out to test the potential associations between skeletal muscle and  
249 circulating levels of IL-15 and IL-15R $\alpha$ , as well as between the former and resistance training  
250 variables, body composition and myofibrillar FSR. The effect of size (ES) was calculated as eta  
251 squared statistic ( $\eta^2$ ) to verify time, condition and between groups differences in systemic and  
252 intramuscular IL-15 and IL-15R $\alpha$  expression. Values are reported as mean  $\pm$  standard deviation  
253 (SD); a  $P < 0.05$  was considered statistically significant.

254

## 255 RESULTS

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

258

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

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

1 268 0.46; Fig. 1C), peaking immediately post-exercise ( $P < 0.001$ ,  $ES = 0.31$ ), and remaining  
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3 269 elevated at 24h post-exercise ( $P = 0.001$ ,  $ES = 0.42$ ).  
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### 7 271 **IL-15R $\alpha$ response to a single dose of resistance exercise.**

8 272 Skeletal muscle mRNA and protein expression levels, and serum IL-15R $\alpha$  concentration in  
9  
10 273 response to a single session of resistance exercise are illustrated in Figure 2. A significant 2-  
11  
12 274 fold increase in IL-15R $\alpha$  mRNA expression above pre-exercise resting values was observed at  
13  
14 275 4h ( $P < 0.001$ ,  $ES = 0.42$ ) and 24h post-exercise ( $P = 0.002$ ,  $ES = 0.35$ ; Fig. 2A). In contrast to  
15  
16 276 IL-15, skeletal muscle protein expression of IL-15R $\alpha$  increased by 1.3-fold ( $P = 0.020$ ,  $ES =$   
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18 277 0.34; Fig. 2B) above pre-exercise resting values at 4h post-exercise, returning to baseline levels  
19  
20 278 24h post-exercise ( $P = 0.036$ ,  $ES = 0.32$ ). Despite of the lack of significant differences between  
21  
22 279 inter-set rest period (1- vs. 5-min groups), the 5-min group tended to show an elevated protein  
23  
24 280 and mRNA expression at 4h and post-exercise ( $\sim 10\%$ ;  $P = 0.103$ ,  $ES = 0.55$  and  $P = 0.092$ ,  $ES =$   
25  
26 281  $0.57$ , respectively; supplementary figure 2). Finally, compared to pre-exercise values, serum  
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28 282 IL-15R $\alpha$  concentration was significantly reduced during the first 60 min following the training  
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30 283 session ( $P < 0.001$ ;  $ES = 0.47$ ; Fig. 2C).  
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### 38 285 **Correlation analysis**

#### 39 286 ***IL-15/IL-15R $\alpha$ and resistance exercise variables.***

40 287 Leg Press 1RM strength was negatively associated with IL-15 serum concentration ( $r = -0.800$ ,  
41  
42 288  $P = 0.003$ ) but not with IL-15R $\alpha$  serum concentration. While the serum IL-15 concentration  
43  
44 289 response to resistance exercise (AUC) was negatively associated with knee extension training  
45  
46 290 volume ( $r = -0.637$ ,  $P = 0.042$ ) and time-under-tension (T-U-T) ( $r = -0.718$ ,  $P = 0.019$ ). Post-  
47  
48 291 exercise (0h), serum IL-15 concentrations were associated with the total volume of knee  
49  
50 292 extension exercise ( $r = -0.934$ ,  $P < 0.001$ ). Furthermore, skeletal muscle protein expression of  
51  
52 293 IL-15R $\alpha$  at 4h post-exercise was associated positively with 1RM Leg press strength ( $r = 0.559$ ,  
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54 294  $P = 0.037$ ) and total training load ( $r = 0.628$ ,  $P = 0.009$ ).  
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4 296 ***Skeletal muscle and circulating expressions of IL-15/IL-15R $\alpha$*** 

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6 297 At baseline, serum IL-15 concentration was positively associated with intramuscular protein IL-  
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8 298 15 levels ( $r = 0.649$ ,  $P = 0.031$ ), but not with mRNA expression. Serum IL-15 immediately  
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10 299 post-exercise was associated with pre-exercise levels of skeletal muscle protein expression of  
11  
12 300 IL-15 and IL-15R $\alpha$  ( $r = 0.582$ ,  $P = 0.037$ ;  $r = -0.599$ ,  $P = 0.031$ , respectively). At baseline, IL-  
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14 301 15 mRNA was associated with IL-15R $\alpha$  mRNA ( $r = 0.592$ ,  $P = 0.043$ ), as well as immediately  
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16 302 ( $r = 0.791$ ,  $P = 0.005$ ) and 24h post-exercise ( $r = 0.653$ ,  $P = 0.021$ ). Additionally, IL-15 and IL-  
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18 303 15R $\alpha$  mRNA expressions at 24h post-exercise was negatively associated with serum  
19  
20 304 concentration of IL-15 and IL-15R $\alpha$  ( $r = -0.620$ ,  $P = 0.042$ ;  $r = -0.727$ ,  $P = 0.005$ ; respectively).  
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25 306 ***Skeletal muscle IL-15/IL-15R $\alpha$  expression and myofibrillar protein synthesis.***

26  
27 307 The myofibrillar fractional synthetic rate (FSR) increased by ~2-fold above resting values from  
28  
29 308 0-4h post-exercise (McKendry et al., 2016) and was associated with IL-15R $\alpha$  mRNA levels at  
30  
31 309 baseline ( $r = 0.662$ ,  $P = 0.019$ ), 4h ( $r = 0.612$ ,  $P = 0.029$ ) and 24h post-exercise ( $r = 0.627$ ,  $P =$   
32  
33 310  $0.029$ ) (Figure 3). Moreover, the delta changes ( $\Delta$ ), from pre-exercise to 4h post-exercise, of IL-  
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35 311 15R $\alpha$  mRNA expression and FSR showed a tendency to be associated at 4h post-exercise ( $r =$   
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37 312  $0.481$ ;  $P = 0.096$ ). No association was observed between myofibrillar FSR and skeletal muscle  
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39 313 IL-15 mRNA or muscle protein expression of either IL-15 or IL-15R $\alpha$  at any time.  
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45 315 **DISCUSSION**

46  
47 316 The present study demonstrates that the gene and protein expression of IL-15R $\alpha$  is up-regulated  
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49 317 in skeletal muscle after a single session of resistance training. The increase in myofibrillar  
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51 318 protein synthesis during 0-4h post-exercise was associated with the expression of IL-15R $\alpha$   
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53 319 mRNA at 4h, which occurred concomitantly with an increase of skeletal muscle IL-15R $\alpha$   
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55 320 protein levels, suggesting increased translation of the IL-15R $\alpha$  gene. These findings indicate  
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1 321 that IL-15R $\alpha$  could have a role in mediating the increase in myofibrillar protein synthesis  
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3 322 observed in skeletal muscle after a single session of resistance training.  
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5 323 Although, in our previous study we demonstrated that myofibrillar protein synthesis rates  
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7 324 were greater when high volume, moderate-intensity resistance exercise was performed with  
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9 325 long (5-min) compared with short (1-min) inter-set rest duration (McKendry et al., 2016), in the  
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11 326 present study, we did not observe significant differences between groups in circulating or  
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13 327 intramuscular IL-15 and IL-15R $\alpha$  expressions, despite skeletal muscle IL-15R $\alpha$  tended to be  
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15 328 elevated in the 5-min compared to the 1-min group (supplementary figure 2). This lack of  
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17 329 differences could be interpreted as evidence to refute the association between skeletal muscle  
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19 330 IL-15/IL-15R $\alpha$  and MPS. Nevertheless, the effect sizes and statistical outputs ( $P < 0.10$ ) indicate  
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21 331 that a potential difference between groups may actually exist. This suggestion is also supported  
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23 332 by the fact that the association between IL-15R $\alpha$  and MPS in the early recovery phase (0-4h  
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25 333 post-exercise) was observed in each group separately (1-min group,  $r = 0.592$ ,  $P = 0.052$ ; and 5-  
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27 334 min,  $r = 0.684$ ,  $P = 0.043$ ). Therefore, our results provide the framework for future studies to  
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29 335 further clarify whether the IL-15/IL-15R $\alpha$  response to strength training reported here have a  
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31 336 physiologically relevant role in human skeletal muscle adaptation to this type of exercise.  
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38 338 The interleukin-15 subunit alpha-receptor (IL-15R $\alpha$ ) is a key subunit receptor of IL-15  
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40 339 that regulates its signalling in several cell types (Budagian et al., 2006; Dubois et al., 2002;  
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42 340 Duitman et al., 2008; Sato et al., 2007). In addition to the common receptor-binding functions,  
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44 341 IL-15R $\alpha$  has been shown to function by itself, without the need for IL-15 binding (Loro et al.,  
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46 342 2015; O'Connell et al., 2015; Pistilli et al., 2011; Pistilli et al., 2013). Animal experiments have  
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48 343 shown that IL-15R $\alpha$  is necessary to maintain insulin sensitivity, since mice lacking IL-15R $\alpha$  are  
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50 344 hyperglycemic and insulin-resistant, despite increased oxidative capacity and reduced fat mass  
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52 345 (Loro et al., 2015). Furthermore, gene-deletion of IL-15R $\alpha$  in mice is accompanied by enhanced  
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54 346 fatigue resistance and a glycolytic-to-oxidative shift in muscle phenotype (O'Connell et al.,  
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56 347 2015; Pistilli et al., 2011). It has been demonstrated that strength training promotes a muscle  
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1 348 myosin heavy chain expression shift from IIx to IIa (Andersen et al., 2005; Campos et al., 2002;  
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3 349 Pareja-Blanco et al., 2016), increasing fatigue resistance. However, it remains unknown  
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5 350 whether IL-15R $\alpha$  up-regulation contributes to this shift in fiber types (from IIx to IIa) with  
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7 351 training in humans. In support of this notion, those participants with a higher IL-15R $\alpha$  protein  
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9 352 expression at 4h post-exercise in our study, performed a greater volume of resistance exercise  
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11 353 and had a higher baseline leg press 1RM. Thus, the up-regulation of IL-15R $\alpha$  could serve as an  
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13 354 adaptive response to maintain muscle characteristics associated with force production. Indeed,  
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15 355 others have reported that two SNPs in exon 7 and 4 of the IL-15R $\alpha$  were able to explain a ~11%  
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17 356 of the hypertrophy observed after 10 weeks of whole-body resistance exercise in 157 young  
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19 357 adults (Riechman et al., 2004). Similarly, another two SNPs, rs2296135 and rs22228059, have  
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21 358 been associated with isometric strength and muscle volume before and after 12 weeks of  
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23 359 unilateral elbow flexor-extensor resistance exercise (Pistilli et al., 2008).

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25 360 Overexpression of IL-15 has revealed that the anabolic/anti-atrophic action of this  
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27 361 interleukin is associated with decreased skeletal muscle proteolysis (Busquets et al., 2005) and  
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29 362 apoptosis through suppression of DNA fragmentation via tumour necrosis factor alpha (TNF- $\alpha$ )  
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31 363 signalling (Figueras et al., 2004). Moreover, IL-15R $\alpha$  mRNA expression is reduced with aging,  
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33 364 and may underpin skeletal muscle atrophy in mice (Marzetti et al., 2009). In agreement, we  
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35 365 have observed an association between IL-15R $\alpha$  mRNA and MPS in the early recovery phase  
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37 366 following resistance exercise. Interestingly, concomitant with the elevation of MPS and IL-  
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39 367 15R $\alpha$  mRNA expression, an up-regulation of IL-15R $\alpha$  protein was also found at 4h post-  
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41 368 exercise, suggesting that IL-15R $\alpha$  may have a role in the induction or maintenance of the  
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43 369 anabolic stimulus during the early post-exercise recovery phase, potentially counteracting the  
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45 370 degree of protein breakdown (Phillips et al., 1997). However, further studies are required to  
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47 371 delineate the role of IL-15R $\alpha$  in exercise-induced muscle remodelling.

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49 372 In contrast to the response observed in skeletal muscle, serum IL-15R $\alpha$  was slightly  
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51 373 reduced at 60 min post-exercise. The discordance between the circulating and skeletal muscle  
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53 374 IL-15R $\alpha$  response to resistance exercise could imply a counteracting mechanism, by which IL-

1 375 15R $\alpha$  binding of IL-15, in blood or cell membrane, reduces its availability (Rubinstein et al.,  
2 376 2006; Schluns et al., 2005) and potentially allows its reabsorption and subsequent restoration of  
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4 377 the intracellular pool of free IL-15.  
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8 378 Although pioneer cell culture studies reported an anabolic effect of IL-15 (Quinn et al.,  
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10 379 2002; Quinn et al., 1997; Quinn et al., 1995), this has not been confirmed in humans. Strength  
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12 380 training-induced muscle hypertrophy is limited to the trained muscles, implying that the  
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14 381 anabolic action of IL-15 in human skeletal muscle cannot be explained by an increase in the  
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16 382 circulating fraction of this myokine. In fact, human experiments do not give support to an  
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18 383 anabolic action of IL-15 in skeletal muscle (Nielsen et al., 2007; Riechman et al., 2004).  
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20 384 However, the physiological relevance of the 24h post-exercise elevation of IL-15 gene  
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22 385 expression in response to resistance exercise, as reported in the present and previous studies  
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24 386 (Nielsen et al., 2007), although suggestive of a role of IL-15 in exercise-induced skeletal muscle  
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26 387 adaptation, remains largely unexplained. The lack of association found between the increase in  
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28 388 IL-15 mRNA at 24h post-exercise and MPS does not support a critical anabolic role, but to  
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30 389 definitely rule out such effect would require the utilization of IL-15 blockers or antibodies,  
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32 390 which cannot be used in humans due to potentially intolerable immunological side effects.  
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34 391 Moreover, the anti-atrophic effect of IL-15 in skeletal muscle has not been tested in the present  
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36 392 study and cannot be excluded (Busquets et al., 2005; Marzetti et al., 2009).  
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42 394 Interleukin-15 is currently considered as a myokine (Grabstein et al., 1994; Quinn et al.,  
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44 395 1995). In agreement, we have observed a positive association between serum concentration of  
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46 396 IL-15 and IL-15 protein levels in skeletal muscle, suggesting that muscle may be an important  
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48 397 source of IL-15 in the basal state. In the present study, we observed that basal IL-15 protein  
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50 398 levels in skeletal muscle were associated with serum concentration immediately post-exercise,  
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52 399 also suggesting that the size of the intramuscular pool could determine the magnitude of the  
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54 400 increase in serum IL-15 elicited by resistance exercise. Nevertheless, the physiological  
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1 401 relevance of the elevated blood IL-15 concentration in close proximity to the end of exercise  
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3 402 remains to be elucidated (Riechman et al., 2004; Tamura et al., 2011).

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5 403 Interestingly, we found that a lower time-under-tension and a lower amount of total  
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7 404 weight lifted during resistance exercise session were associated with higher post-exercise serum  
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9 405 IL-15 concentration, which could indicate that a more prolonged muscle activation may  
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11 406 attenuate the release of IL-15. Depletion of the intracellular pool or reduced *de novo* synthesis  
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13 407 of IL-15 could explain the attenuated release of IL-15 with greater training load, given the short  
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15 408 life of IL-15 in plasma (Rubinstein et al., 2006; Stoklasek et al., 2006).

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18 409 The fact that the increase of circulating IL-15 was not accompanied by an increase in its  
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20 410 soluble receptor implies that after resistance training there is more free IL-15, available to act  
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22 411 on target tissues (Mortier et al., 2004). IL-15 is a potent pro-inflammatory cytokine that  
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24 412 stimulates proliferation, maturation and has protective effects on several immune cells  
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26 413 (Budagian et al., 2006). In addition to the immunological effects, IL-15 has anti-adipogenic  
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28 414 effects in rodents (Carbo et al., 2001). Therefore, although the physiological role of the  
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30 415 systemic elevation of IL-15 in response to strength training remains unknown, immunological  
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32 416 and metabolic effects are possible.

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38 418 In conclusion, the IL-15/IL-15R $\alpha$  signalling pathway is activated in human skeletal  
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40 419 muscle in response to a single session of resistance exercise. Skeletal muscle mRNA levels and  
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42 420 protein IL-15R $\alpha$  expression were elevated four hours after resistance exercise and were  
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44 421 positively associated with increased rates of myofibrillar protein synthesis. Therefore, as  
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46 422 previously shown in cell culture and *in vivo*, the present investigation lends support to a  
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48 423 potentially anabolic effect of IL-15R $\alpha$  in human skeletal muscle. Moreover, our experimental  
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50 424 results indicate that IL-15 and IL-15R $\alpha$  may play a role in exercise-induced muscle  
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52 425 remodelling. Prolonged resistance training studies are necessary to determine the relevance of  
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54 426 IL-15R $\alpha$  in muscle protein synthesis/breakdown, as well as the precise role of circulating and  
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56 427 muscular levels of IL-15 and its receptor IL-15R $\alpha$  in chronic physiological adaptations.  
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4 429 **Perspectives**

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6 430 Previous studies have suggested a role of IL-15R $\alpha$  in muscle phenotypic adaptation to  
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8 431 resistance training. The present study confirms the activation of IL-15/IL-15R $\alpha$  signalling  
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10 432 pathway in human skeletal muscle in response to a single session of resistance exercise. It  
11  
12 433 remains to be determined how skeletal muscle contributes to circulating levels of IL-15 and  
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14 434 how circulating IL-15 could influence skeletal muscle and adipose tissue mass. Given the  
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16 435 important role that IL-15 has in immune responses, the link between physical activity, skeletal  
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18 436 muscle IL-15 production and immunity deserves further attention. The fact that IL-15R $\alpha$  is  
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21 437 independently associated with myofibrillar protein synthesis and muscle phenotype implies that  
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23 438 the axis IL-15/ IL-15R $\alpha$  may have an important role in human skeletal muscle remodelling.  
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17  
18          447   Blot analysis.

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23          449   **CONFLICT OF INTERESTS**

24  
25          450   All the authors declare that they have no conflict of interest derived from the outcomes of this  
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27          451   study.

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31          453   **AUTHOR CONTRIBUTIONS**

32  
33          454   APL, JM and LB conceived and designed the experiment. APL, JM and LB collected the data.  
34  
35          455   APL, JM, MMR, DMA, BPK, DV, JB, JALC and LB analysed and interpreted the data. APL,  
36  
37          456   JALC and LB drafted the manuscript and prepared all figures. All authors read and approved  
38  
39          457   the final version of the manuscript.

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44          459   **REFERENCES**

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1 564 **FIGURE LEGENDS**

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6 566 **Figure 1.** IL-15 response to a single session of resistance exercise.

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8 567 IL-15 mRNA (qRT-PCR) (A) and protein levels (Western blotting) (B) from *vastus lateralis*  
9 568 muscle biopsies, and serum IL-15 levels (ELISA) (C). Values are presented as means  $\pm$  SD (N  
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13 569 = 14). \*  $P < 0.05$  compared to Pre-exercise. #  $P < 0.05$  compared to 0h post-exercise. Data were  
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15 570 log-transformed.

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20 572 **Figure 2.** IL-15R $\alpha$  response to a single session of resistance exercise.

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22 573 IL-15R $\alpha$  mRNA (qRT-PCR) (A) and protein levels (Western blotting) (B) from *vastus lateralis*  
23 574 muscle biopsies, and serum IL-15R $\alpha$  levels (ELISA) (C). Values are presented as means  $\pm$  SD  
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27 575 (N = 14). \*  $P < 0.05$  compared to Pre-exercise. #  $P < 0.05$  compared to 0h post-exercise.  $\Phi$   
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29 576  $P < 0.05$  compared to 24h post-exercise.  $\S P < 0.05$  differences compared to Mid-exercise. Data  
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31 577 were log-transformed.

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36 579 **Figure 3.** Relationship between myofibrillar protein synthesis measured as a fractional  
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38 580 synthetic rate (FSR) and IL-15R $\alpha$  mRNA pre- and post-exercise. The association remained  
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40 581 significant in Fig. 3B ( $r = 0.665$ ,  $P = 0.026$ ) when the lowest FSR values were excluded. a.u.,  
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42 582 arbitrary units.

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47 584 **Supplementary Figure 1.** Skeletal muscle IL-15 response to a single session of resistance  
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49 585 exercise by group (1 and 5 min groups).

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51  
52 586 IL-15 mRNA (qRT-PCR) (A) and protein levels (Western blotting) (B) from *vastus lateralis*  
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54 587 muscle biopsies. Values are presented as means  $\pm$  SD (N = 14). \*  $P < 0.05$  compared to Pre-  
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56 588 exercise. Data were log-transformed. a.u., arbitrary units.

1           589   **Supplementary Figure 2.** Skeletal muscle IL-15R $\alpha$  response to a single session of resistance  
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3           590   exercise by group (1 and 5 min groups).  
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6           591   IL-15R $\alpha$  mRNA (qRT-PCR) (A) and protein levels (Western blotting) (B) from *vastus lateralis*  
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8           592   muscle biopsies. Values are presented as means  $\pm$  SD (N = 14). \* P<0.05 compared to Pre-  
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10          593   exercise. # P<0.05 compared to 0h post-exercise. Data were log-transformed. a.u., arbitrary  
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12          594   units.  
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PROOF

**Table 1.** Participants' physical characteristics

N	14
Age (yrs)	24.9 ± 4.8
Body mass (kg)	82.2 ± 11.9
BMI (kg/m <sup>2</sup> )	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.

1 **Table 2.** Primers for qRT-PCR analysis.

Primer	Sequence	Accession Number	T <sub>m</sub>
IL-15	F: 5'-AAAGTGATGTTACCCCAGTTG	NM_000585.4	60°
	R: 3'-CCTCCAGTTCCTCACATTCTTTG		30s
IL-15R $\alpha$	F: 5'-CAGCCGCCAGGTGTGTATC	NM_002189.3	60°
	R: 3'-TTGCCTTGACTTGAGGTAGCA		30s

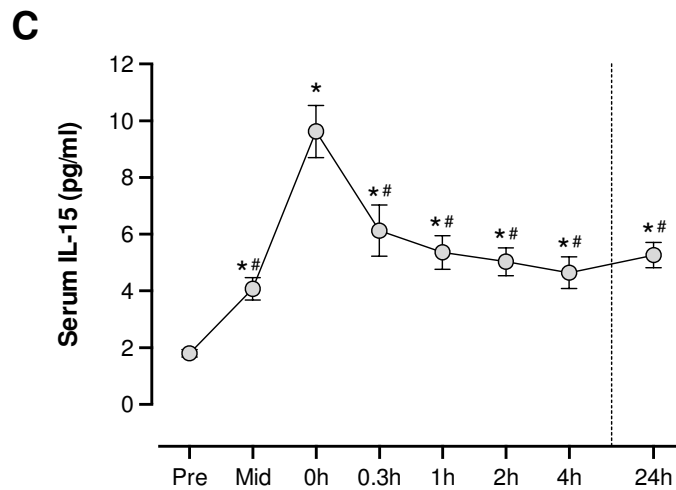
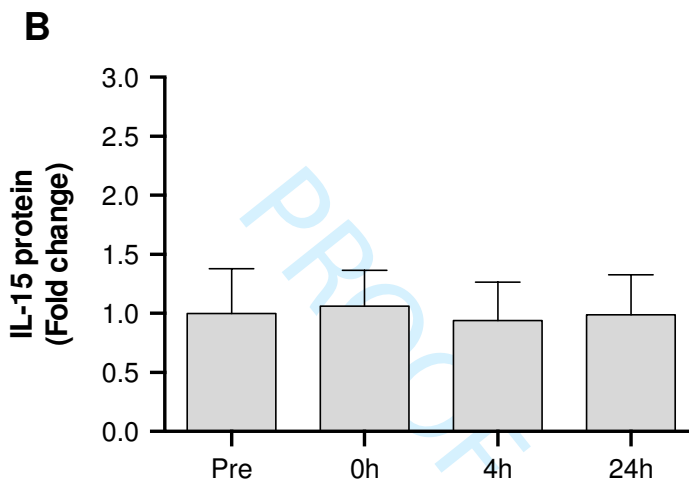
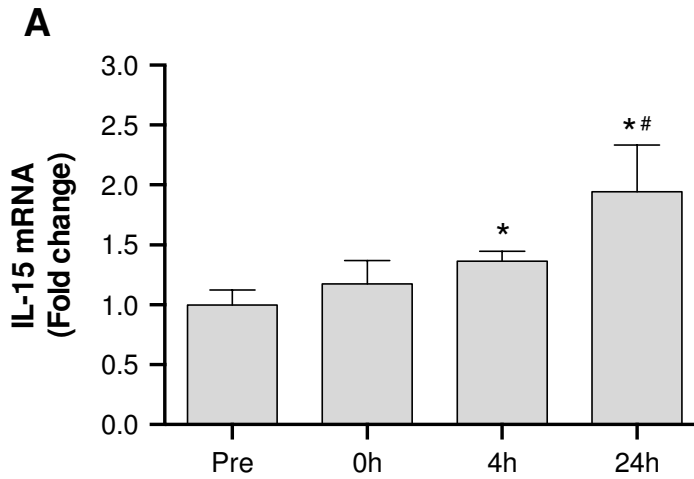
2 T<sub>m</sub>, melting temperature.

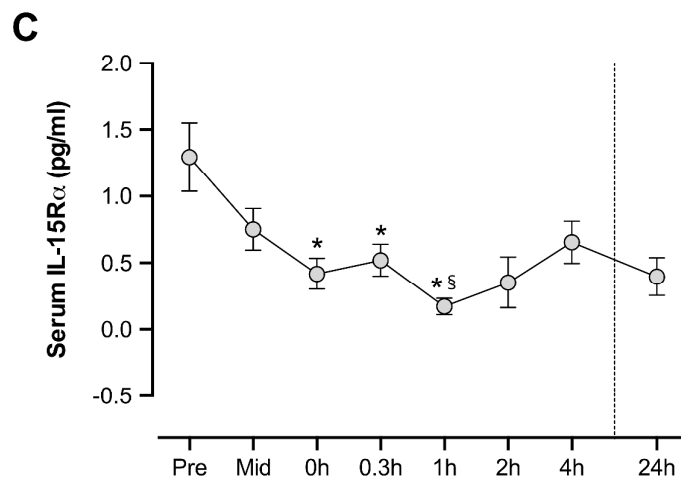
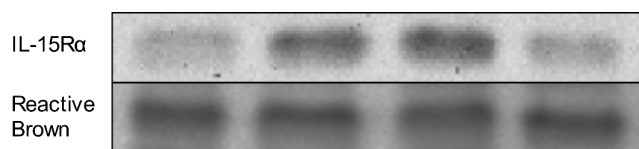
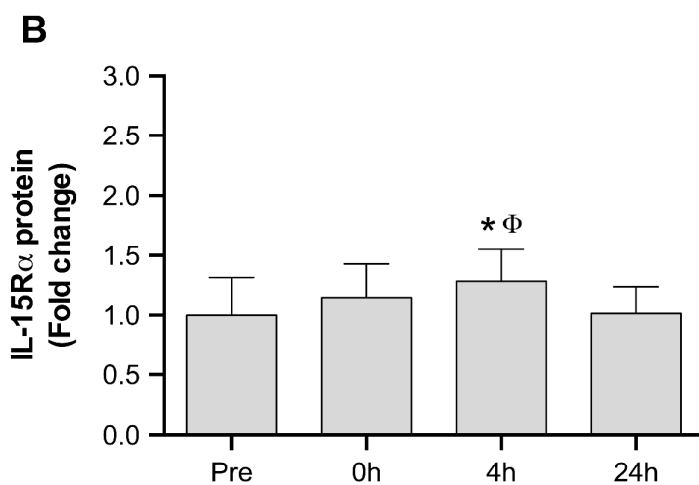
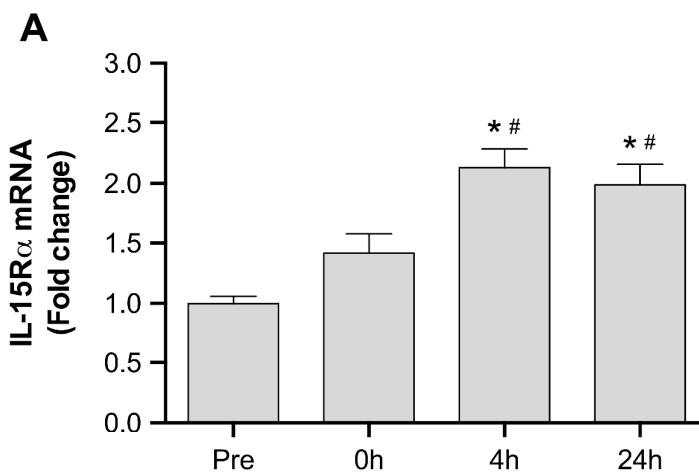
PROOF

1 **Table 3.** Variables describing the resistance session.

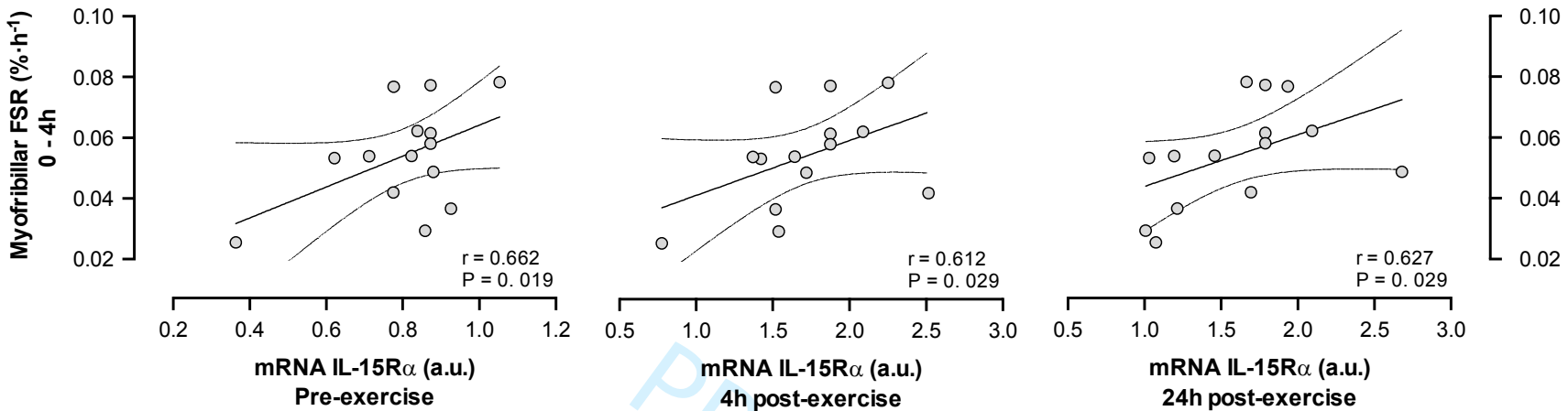
Set	Leg Press					Knee Extension					Total
	1	2	3	4	Total	1	2	3	4	Total	8
Load (kg)	205±40	205±39	205±38	206±37	820±154	123±18	119±19	117±19	114±18	473±73	1293±210
Repetition	13±3	11±2	11±2	10±2	44±6	9±2	9±2	10±2	9±2	37±6	10±1
Volume (kg)	2484±401	2188±296	2260±452	2019±533	8951±1217	1068±276	1119±296	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

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



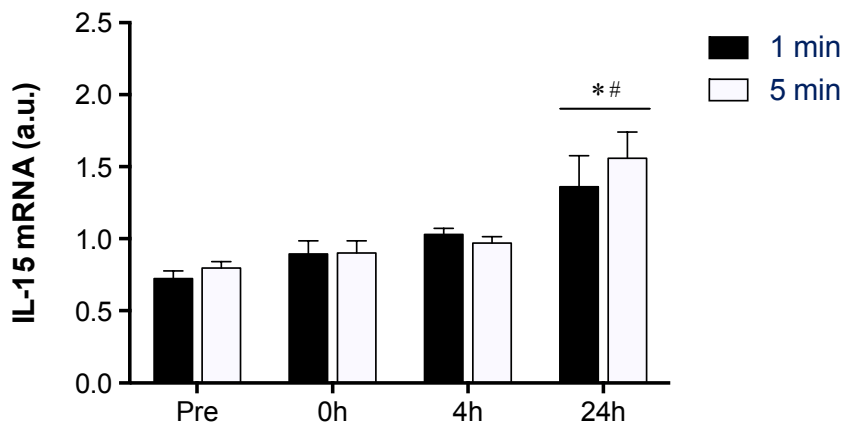






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**A**



**B**

