Association of interleukin-15 protein and interleukin-15 receptor genetic variation with resistance exercise training responses

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Riechman, Steven E., G. Balasekaran, Stephen M. Roth, and Robert E. Ferrell. Association of interleukin-15 protein and interleukin-15 receptor genetic variation with resistance exercise training responses. J Appl Physiol 97: 2214–2219, 2004; doi:10.1152/japplphysiol.00491.2004.—Interleukin-15 (IL-15) is an anabolic cytokine that is produced in skeletal muscle and directly affects muscle anabolism in animal and in vitro models. The contribution of IL-15 variability in muscle responses to 10 wk of resistance exercise training in young men and women was examined by measuring acute and chronic changes in IL-15 protein in plasma and characterizing genetic variation in the IL-15 receptor-α gene (IL15RA). Participants trained 3 days a week at 75% of one repetition maximum, performing three sets (6–10 repetitions) of 13 resistance exercises. Plasma IL-15 protein was significantly increased ($P < 0.05$) immediately after acute resistance exercise but did not change with training and was not associated with variability in muscle responses with training. A single nucleotide polymorphism in exon 7 of IL15RA was strongly associated with muscle hypertrophy and accounted for 7.1% of the variation in regression modeling. A polymorphism in exon 4 was also independently associated with muscle hypertrophy and accounted for an additional 3.5% of the variation in hypertrophy. These results suggest that IL-15 is an important mediator of muscle mass response to resistance exercise training in humans and that genetic variation in IL15RA accounts for a significant proportion of the variability in this response.

IL-15; strength; skeletal muscle; muscle quality

RESISTANCE EXERCISE TRAINING (RET) is well known to result in marked increases in muscle mass and strength, but the responses to a standardized program are considerably variable among individuals (19). Our understanding of the characteristics that account for this interindividual variability in muscle responses is limited. The Molecular Epidemiology of Resistance Exercise Training (MERET) study was designed to examine environmental and genetic contributions to these variable muscle responses in young men and women. The present report examined the association of genetic variation in the IL-15 receptor-α to muscle responses to 10 wk of high-intensity resistance training as well as the plasma changes in IL-15 protein in response to acute and chronic resistance exercise.

IL-15 was initially identified as a T-cell growth factor and shares many properties and functions with IL-2 (10), including sharing two of three components of their heterotrimeric receptor complexes (IL-2 Rβ and IL-2 Rγc). Unlike IL-2, however, IL-15 signals through the IL-15 receptor-α and is found in a wide variety of tissues including skeletal muscle. In fact, skeletal muscle levels of IL-15 are among the highest of any tissue (11, 17), and IL-15 is one of the most abundant cytokines in skeletal muscle (15).

IL-15 has been shown to be anabolic for differentiated mouse C2 and cultured bovine primary muscle cells marked by an increase myosin heavy chain accumulation (18). In a rat model of cancer cachexia, IL-15 treatment inhibited skeletal muscle wasting by decreasing protein degradation rates through inhibition of the ATP-ubiquitin-dependent proteolytic pathway (6). Similarly, transfection of C2 cells with an IL-15 expression vector induced myotube hypertrophy, independent of IGF action, through increases in protein synthesis and decreases in protein degradation (16). The anabolic properties in these models did not include any effect of IL-15 on proliferation or differentiation of myocytes, although inhibition of IGF, through a stable transfection of IGF binding protein, resulted in elevated differentiation with IL-15 administration to C2 cell cultures (17).

The anabolic effects of IL-15 on skeletal muscle suggest a promising treatment for muscle loss conditions such as cachexia, sarcopenia, and disuse atrophy. However, existing studies have been limited to animal and in vitro models, and no study has addressed the role of IL-15 signaling on variability in muscle phenotypes in humans. Therefore, a primary purpose of this investigation was to determine whether genetic variation in the IL-15 receptor-α gene or plasma IL-15 concentrations were associated with the variability in muscle mass, strength, and muscle quality changes after standardized high-intensity RET.

MATERIALS AND METHODS

Subjects. One hundred fifty-three participants completed 10 wk of RET (76 men and 77 women). Eligibility was based on the following criteria: age between 18 and 31 yr; no medical history for disorders involving the cardiovascular, endocrine, or skeletal muscle systems; <3 h of RET activity per week in the past year; normal caloric consumption; and no report of taking anabolic steroids or other putative performance-enhancing supplements. Participants were instructed not to perform RET activity outside of the program and to maintain all other non-RET activities at the same level. After all procedures were explained, subjects gave written, informed consent before participation in the study under protocols approved by the University of Pittsburgh Institutional Review Board. A more detailed description of the cohort and the intervention has been reported (19).

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One repetition maximum. Three familiarization-training sessions were conducted before the initiation of the program, using the minimum weight to induce resistance. After these familiarization sessions, one repetition maximums (1 RMs) were determined after a 3-min warm-up on an aerobic exercise machine and stretching. Before a 1 RM attempt, subjects performed three warm-up repetitions at a weight equivalent to 50% of an estimated 1 RM. The weight was then increased to 90% of a reestimated 1 RM to perform one repetition. After 60 s of rest, the weight was increased to 100% for a 1 RM attempt. Thereafter, successful attempts were followed by attempts made with a higher resistance in a manner that minimized the total number of attempts required before the 1 RM value was obtained. This procedure was performed on all exercise equipment used for the RET program in the same order for all participants within 4 days of starting and 4 days of completing the RET.

Body composition. Baseline and post RET assessments were conducted for height (wall-mounted stadiometer), weight (Detecto Clinica scale), body composition by hydrostatic weighing (21), and circumference measurements of arms and thighs. All measurements were made with subjects in the minimal attire used to enter the underwater weighing tank within 2–4 days of starting or completing the RET. Arm circumference was measured at the middle of the biceps with the arm relaxed at the side of the body. Thigh circumference was measured at the midpoint of the femur. Duplicate measures were made of each circumference and recorded to the nearest 0.1 cm.

RET. The 10-wk, three sessions/week RET program included 13 exercises encompassing all major muscle groups with weights set at 80% of 1 RM. These sessions were supervised by exercise physiologists (participant-to-trainer ratio 2:1 to 3:1). The RET program included the following exercises on the Strive (Canonsburg, PA) weight machines: chest press, seated row, lateral pulldowns, leg extensions, triceps extension, arm curl, shoulder press, hamstring curl, low back extension, abdominal crunch, and incline press. Leg press and calf raises were performed on a Universal leg press machine. A warm-up consisting of exercise on an aerobic machine for 5 min followed by stretching was performed before each day of RET. Subjects performed three sets of 6–10 repetitions for each exercise. Subjects were given 30 s of rest between each set and 1 min between exercises. Resistance settings were increased when participants were able to complete 10 repetitions on a particular set to maintain the relative difficulty at the same level for all subjects throughout the study.

Genetic analysis. Genomic DNA from 12 control samples of diverse ethnic background were used to search for genetic variation in the IL-15 receptor gene (IL15RA). Primers were selected so as to cover all exonic regions and exon-intron borders using cDNA sequence found in GenBank (accession no. U31628). After amplification of these regions by PCR (35 cycles), fragments were directly sequenced using the dRhodamine ready reaction kit (Perkin-Elmer) and analyzed on the ABI Prism model 377 fluorescent sequencer. Sequences were aligned for comparison by use of Sequencher 3.0 (Gene Codes). Variations were confirmed by restriction enzyme digestion of amplicons when more than one of the 24 chromosomes indicated a sequence variant. Polymorphisms in exon 4, exon 5 intron-exon border, and exon 7 were subsequently genotyped in the MERET cohort. Methods for other regions are reported in supplementary material (Jap.physiology.org/cgi/content/full/00491.2004/DC1).

Genomic DNA was extracted from the buffy coat from blood samples by standard methods (14). The exon 4 variant (C to A) results in Thr/Asn amino acid change (SNP accession rs3136617) and was genotyped by using the enzyme BstNI. The exon 5 intron-exon border variant (A to G) is 20 bp from the border in intron 5 (SNP accession rs3136618) and was genotyped by using the enzyme HpaII. The exon 7 variant (A to C) is in the 3’ untranslated region (SNP accession rs2296135) and was genotyped by using the enzyme PstI. To create a PsfI restriction site, it was necessary to insert a mismatch in the forward primer.

For the exon 7 PsfI variation, MERET samples were amplified by using forward (ctt ccc tcc ctc atc ata cC c) and reverse (cca ggt ccc tgc cca ggt gtc) primers. Capital C indicates the mismatch. Each 35-cycle PCR reaction was performed using 54°C annealing temperature and 50-μl final reaction volumes containing 100–200 ng DNA, 0.46 μM of deoxyribonucleotide triphosphate (dNTP) mixture, 50 mM KCl, 10 MM Tris-HCl (pH = 9.0), 1.5 mM MgCl, 0.1% Triton X-100, and 0.8 U of Taq DNA polymerase.

Amplified product (16 μl) from study subjects was added to 5 U of PstI for digestion overnight at 37°C. Each digested sample was loaded onto a 2.5% agarose gel containing ethidium bromide and electrophoresed for 3.5 h at 100 V. Internal controls were run on each of these gels to ensure complete digestion by the PstI enzyme. After electrophoresis, the DNA fragments were visualized by ultraviolet illumination (Eagle Eye), and fragment sizes were estimated by comparison to a 1-kb ladder run on the same gel. The presence of a polymorphic restriction site (PstI) at the IL15RA gene locus is specified as C base, whereas absence of this site is an A base. Subjects were categorized as exhibiting the CC, CA, or AA genotype.

The same methods were used for the exon 4 BstNI and intron 5 HpaII sites with the following exceptions. The fragments containing the BstNI and HpaII sites were amplified with 50°C annealing temperature and the primers F: tga att ctc ctt ggg cct c and R: gct ggt cgg gac tca t and F: atc tga tgg agg cct tct gag R: gct tga gga gga act agg acc, respectively. Digestion using the BstNI enzyme requires 60°C incubation. Presence of the restriction site for the BstNI or HpaII sites indicated a C allele. Subjects were categorized as exhibiting CC, CA, or AA genotype for BstNI and AA, AG, or GG for HpaII. Most CC genotypes for the BstNI site corresponded to the AA genotype for the HpaII site. Likewise, CA and AA (BstNI) corresponded to AG and GG (HpaII), respectively. Therefore, C:A and A:G (BstNI:HpaII) haplotypes were assigned as well.

IL-15 protein analysis. Blood samples were collected in 10 ml EDTA (Vacutainer) before the first and last RET session after a minimum 8-h fast. A second blood draw was collected immediately after (within 5 min) the first and last RET session. Samples were immediately placed on ice, centrifuged, and stored at −70°C in 1-ml aliquots. Plasma samples were assayed for concentrations of IL-15 using the QuantiGlo human IL-15 Immunoassay kit method (R&D Systems, Minneapolis, MN). The detection range for IL-15 is 0.3 to 30 pg/ml. The inter- and intra-assay coefficients of variation for each assay were <10% throughout the assays (mean <5%).
were performed to test the independent association of IL15RA genotypes on lean mass response to RET. A repeated-measures ANOVA was used to examine differences in IL-15 protein at the beginning and end of training and response to acute RET. Change in hematocrit was used as a covariate to control for plasma volume shifts with exercise. Pearson’s correlation coefficients were calculated to estimate the association of IL-15 protein concentrations to muscle responses to RET.

Percent gains were calculated as 100 \times (\text{postmeasurement} - \text{premeasurement})/\text{premeasurement}. Arm strength was calculated as 1 RM biceps kg + 1 RM triceps kg. Arm muscle quality (MQ) was calculated as arm MQ = (1 RM biceps kg + 1 RM triceps kg)/arm circumference (cm). Leg strength was calculated as 1 RM hamstring curl kg + 1 RM leg extension kg. Composite strength for arm and leg was reported as summary of strength measures that was consistent for all measures of strength in this study and specifically in this report because these composite scores were used in the calculation to estimate muscle quality. Leg muscle quality was calculated as leg MQ = [(1 RM hamstring curl kg + 1 RM leg extension kg)/(thigh circumference cm)].

RESULTS

Subjects. The baseline characteristics stratified by IL15RA PstI genotype of the 76 men and 77 women who completed the training are presented in Table 1. Of these subjects, 14 (9.3%) of those who completed the training were not Caucasian (i.e., African American, Hispanic, Asian). Baseline characteristics were similar between genotype groups \((P > 0.05)\) including HpaII and BstNI genotypes (data not shown). Frequencies of the genotypes were in Hardy-Weinberg equilibrium and were similar between genotype groups \((P > 0.05)\). These observations were significant in both men and race categories \((P < 0.05)\) including African-American participants.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CC</th>
<th>CA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women (n)</td>
<td>24/22</td>
<td>38/36</td>
<td>14/19</td>
</tr>
<tr>
<td>Age, yr</td>
<td>21.1 ± 2.4</td>
<td>20.9 ± 2.6</td>
<td>20.7 ± 2.4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171.6 ± 9.6</td>
<td>170.3 ± 8.4</td>
<td>169.5 ± 9.7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.7 ± 15.0</td>
<td>72.3 ± 15.0</td>
<td>72.7 ± 16.3</td>
</tr>
<tr>
<td>Fat, %</td>
<td>23.1 ± 6.1</td>
<td>22.3 ± 7.7</td>
<td>23.4 ± 8.8</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>57.1 ± 12.1</td>
<td>55.9 ± 11.4</td>
<td>55.3 ± 12.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. Arm strength = triceps extension + biceps curl \(1\) repetition maximum (1 RM), leg strength = hamstring curl + leg extension 1 RM. Arm muscle quality (MQ) = (1 RM biceps + 1 RM triceps)/arm circumference. Leg MQ = (1 RM hamstring curl + 1 RM leg extension)/thigh circumference. *\(P < 0.05\) for genotype (analysis of covariance).

Haplotypes. Haplotype frequencies for the IL15RA BstNI, HpaII, and PstI sites were as follows: C:C:A = 0.355, A:G = 0.296, A:G:C = 0.138, C:A:A = 0.128, A:A:C = 0.025, A:A:A = 0.022, C:G:C = 0.022, and C:G:A = 0.014. The frequencies of the BstNI and HpaII C:A and A:G haplotypes were 0.483 and 0.434, respectively. These BstNI and HpaII haplotypes were used to analyze muscle responses stratified by PstI genotypes (Table 3). The haplotype combination across all three sites can be deduced from the table with the exception of A:C:G/A:CA (BstNI/HpaII/PstI genotypes), which would have either A:G:C/CA:A/A or A:G:A/C:CA haplotype combination.

Muscle mass response and BstNI, HpaII haplotype. The presence of a C:A (BstNI:HpaII) haplotype was associated with a significant \((P < 0.05)\) increase in lean mass (Table 3). The addition of a second C:A had no effect on the change in lean mass. Because data were excluded that did not have the A:G or C:A haplotypes exclusively or that did not have all three genotypes, the sample size was reduced to 118. The effect of the PstI genotypes remained consistent between haplotypes (i.e., there were no interactions). No additional effects of the haplotypes were observed for other muscle response phenotypes (data not shown).

Multiple regression. To determine the independent association of the IL15RA genotypes on muscle mass response to RET, stepwise multiple regression was performed (Table 4). Pst and BstNI genotypes were independently associated with muscle mass response to RET and predicted 7.1 and 3.5% of the gain, respectively. The BstNI/HpaII haplotype variable did not enter the model when BstNI genotypes were submitted as as well. Interactions between genotypes and covariates were also examined but did not significantly add to the model. Only the PstI site was also independently associated with arm and leg circumference and muscle quality gains after adjusting for known covariates (data not shown).

IL-15 protein. Resting/fasting plasma concentrations of the IL-15 protein were significantly increased in response to acute resistance exercise at the first and last (30th) session of the 10 wk of RET, even after adjustment for plasma volume shifts (Fig. 1). No changes were observed in resting/fasting concentrations or acuteness responsiveness from the first to the last training session. There were no differences observed for age or gender for resting concentrations or responsiveness (data not shown); however, African-American participants had signifi-
differences in muscle responses and is merely in linkage the gene. The fact that the variation is not translated suggests genetic variation in the IL-15 receptor-
examined the physiological changes of IL-15 in plasma and growth in animal and cell culture models (6, 17, 18). We (cytoplasmic-signaling domain) in the 3
mass gain independent of the PstI variation. HpaII haplotypes were also significantly associated with lean
gains. Moreover, IL15RA exon 4 (BstNI) genotypes or BstNI/
with greater muscle mass gains but reduced muscle quality
resistance exercise, but this response did not change with the
Plasma IL-15 increased significantly in response to acute
mass, strength, and quality responses to standardized RET.
these factors were associated with the variability in muscle
changes in IL-15 were not significantly correlated to muscle
changes in IL-15. Resting concentrations and acute and chronic
values of these analyses was reduced because of the lack of availability of all four blood
data not shown). The sample size of these analyses was

**DISCUSSION**

IL-15 has been shown to be a strong stimulant for muscle
growth in animal and cell culture models (6, 17, 18). We examined the physiological changes of IL-15 in plasma and
genetic variation in the IL-15 receptor-α to determine whether these factors were associated with the variability in muscle mass, strength, and quality responses to standardized RET. Plasma IL-15 increased significantly in response to acute resistance exercise, but this response did not change with the 10 wk of RET and was not associated with variability in muscle responses to training. However, the exon 7 PstI C to A polymorphism in the IL-15 receptor-α gene was associated with greater muscle mass gains but reduced muscle quality gains. Moreover, IL15RA exon 4 (BstNI) genotypes or BstNI/ HpaII haplotypes were also significantly associated with lean mass gain independent of the PstI variation.

The IL15RA PstI genetic variation is found in exon 7 (cytoplasmic-signaling domain) in the 3’ untranslated region of the gene. The fact that the variation is not translated suggests that this variation may not be responsible for the observed differences in muscle responses and is merely in linkage disequilibrium with a functional site. Examination of IL15RA variation in a SNP database (http://snpper.chip.org) did not reveal any additional variation that would likely alter function of the receptor. However, alternate exon 7 splicing has been observed to cause two distinct exon 7 isoforms within the IL-15 receptor protein, leading to the possibility that genetic variation in the IL-15 receptor mRNA influences the splicing pathway or translation efficiency. The IL-15 receptor has two other splice variants in which exon 2 and exon 3 are deleted, resulting in eight observed isoforms. In the original description of these splice variants, no differences in signaling or binding of IL-15 were observed (2, 9), with the exception of the exon 2 deletion isoform, which does not bind IL-15. The exact role of the PstI variation remains to be determined by functional studies of the gene and protein. No measurements of IL-15 receptor were made in the present study, so no conclusions about the potential influence of IL15RA polymorphisms on receptor isoforms can be proposed.

The exon 4 BstNI Asn to Thr variation would seem to be the most interesting because it leads to an amino acid change to Thr in a Pro-Thr-rich area of the extracellular domain. However, the effects on muscle responses to RET were quite modest compared with the PstI site. Although these two sites were in modest linkage disequilibrium (D’ = 0.402, P < 0.001), their effects were independent in regression modeling. This variation has been reported previously, but these authors did not demonstrate differences in cell proliferation or IL-15 binding (2). This BstNI polymorphism was in very strong linkage disequilibrium with the HpaII site (D’ = 0.875, P < 0.001). Analysis of the haplotypes of these two sites demonstrated a small effect with the addition of one C:T (BstNI: HpaII) variant. The IL15R HpaII variation is found at the

**Table 4. Multiple regression analysis to determine the independent association of IL15 receptor genotypes on lean mass response to 10 wk of RET**

<table>
<thead>
<tr>
<th>DV = change in lean mass, kg</th>
<th>Non-Standardized Regression Coefficient</th>
<th>Standard Error</th>
<th>P Value</th>
<th>R² Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>4.299</td>
<td>1.311</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>IL15 receptor PstI genotype</td>
<td>0.810</td>
<td>0.198</td>
<td>&lt;0.001</td>
<td>0.071</td>
</tr>
<tr>
<td>Age, yr</td>
<td>-0.136</td>
<td>0.056</td>
<td>0.017</td>
<td>0.043</td>
</tr>
<tr>
<td>OC use</td>
<td>-0.460</td>
<td>0.165</td>
<td>0.006</td>
<td>0.039</td>
</tr>
<tr>
<td>RET history, h/wk</td>
<td>-0.221</td>
<td>0.084</td>
<td>0.009</td>
<td>0.042</td>
</tr>
<tr>
<td>IL15 receptor BstNI genotype</td>
<td>-0.470</td>
<td>0.197</td>
<td>0.018</td>
<td>0.035</td>
</tr>
</tbody>
</table>

RET, resistance exercise training. Oral contraceptive (OC) use scores: Men = 0, No OC = 1, OC = 2.

**Fig. 1.** Plasma IL-15 protein concentration before and after resistance exercise at the beginning (session 1) and end (session 30) of the resistance exercise training intervention (N = 124).

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The effects of TNF-α may also play an important role in IL-15-mediated hypertrophy. TNF-α is a catabolic cytokine and has been shown to be elevated in muscle inflammatory disease (23, 25), muscle injury models (8), cachexia (7, 26), and frail elderly and is reduced with resistance training in the frail elderly (12). Higher levels of TNF-α were also associated with lower muscle mass in elderly (28). TNF-α stimulates an important pathway in apoptosis signaling (29), whereas IL-15 can potently inhibit the process (5). Moreover, the IL-15 receptor can specifically block TNF-α-mediated apoptosis by recruiting the TNF receptor-associated factor (TRAF2) and preventing TNF signaling. This may be accomplished by CD40 TRAF2 interacting motifs that are also found in the cytoplasmic tail of the IL15R protein (4). IL-15 receptor signaling may also rescue cells from apoptosis through phosphorylation of IkB and subsequent activation of NF-κB. It may be possible that the genetic variation in the IL-15 receptor affects this particular mechanism of IL-15 action on muscle hypertrophy directly or that isoforms of the IL-15 receptor protect against TNF-mediated apoptosis to different degrees.

In summary, chronic RET induces skeletal muscle hypertrophy as well as increases in strength. However, not every individual can expect the same magnitude of muscle responses to a standard program because of genetic and environmental factors yet to be thoroughly characterized. The effect of IL-15 signaling was examined as a contributing mediator of this interindividual variability in muscle responses by measuring IL-15 protein in plasma during exercise and training and characterizing genetic variation in the IL15RA gene. Plasma IL-15 was increased immediately after exercise but did not change with training and was not associated with muscle responses to training. A genetic variation in the 3’ untranslated region of the IL15RA gene was strongly associated with muscle hypertrophy, although those with the greatest hypertrophy had lower muscle strength and muscle quality increases. Another genetic variation that resulted in the addition of a Thr in the Pro- and Thr-rich area of the IL-15 receptor was weakly associated with muscle hypertrophy. Taken together with other studies of IL-15 on muscle anabolism, this study suggests that IL-15 is an important mediator of muscle phenotypes in humans.

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