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INTERLEUKIN-15 AND INTERLEUKIN-15R α SNPs AND ASSOCIATIONS WITH MUSCLE, BONE, AND PREDICTORS OF THE METABOLIC SYNDROME

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Abstract

The aims of this study were to examine associations between two SNPs in the human IL-15 gene and three SNPs in the IL-15R α gene with predictors of metabolic syndrome and phenotypes in muscle, strength, and bone at baseline and in response to resistance training (RT). Subjects were Caucasians who had not performed RT in the previous year and consisted of a strength cohort (n=748), volumetric cohort (n=722), and serum cohort (n=544). Subjects completed 12 weeks of unilateral RT of the non-dominant arm, using their dominant arm as an untrained control. ANCOVA analyses revealed gender-specific associations with: 1) IL-15 SNP (rs1589241) and cholesterol (p=0.04), LDL (p=0.02), the homeostasis model assessment (HOMA; p=0.03), and BMI (p=0.002); 2) IL-15 SNP (rs1057972) and the pre- to post-training absolute difference in 1RM strength (p=0.02), BMI (p=0.008), and fasting glucose (p=0.03); 3) IL-15R α SNP (rs2296135) and baseline total bone volume (p=0.04) and the pre- to post-training absolute difference in isometric strength (p=0.01); and 4) IL-15R α SNP (rs2228059) and serum triglycerides (p=0.04), baseline whole muscle volume (p=0.04), baseline cortical bone volume (p=0.04), and baseline muscle quality (p=0.04). All associations were consistent in showing a potential involvement of the IL-15 pathway with muscle and bone phenotypes and predictors of metabolic syndrome.

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Keywords

polymorphisms; cytokines; metabolic syndrome

1. Introduction

Interleukin-15 (IL-15) is a recently discovered cytokine that belongs to the four α -helix bundle family of cytokines, which also include IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and IL-9 (for in-depth reviews on IL-15, see [1,2]). Within the immune system, IL-15 can stimulate the proliferation of T cells [3], aid in the maintenance of memory CD8⁺ T cells [4,5], activate natural killer (NK) cells [6], and stimulate proliferation of B cells [7]. Interleukin-15 mRNA is widely distributed among tissue and cell types, including heart, brain, lung, liver, kidney, pancreas, skeletal muscle, and macrophages [3]. Additionally, among cytokine mRNAs expressed within skeletal muscle, IL-15 mRNA has been reported to be the highest [8,9]. The IL-15 receptor is a trimeric structure composed of the IL-2R/IL-15R beta-chain, the common gamma (γ) chain, and a receptor specific alpha-chain (IL-15R α) [10]. Recent studies have demonstrated that signaling via IL-15 can occur not only through binding to its trimeric receptor but also through a complex formed between IL-15 and the IL-15R α [11,12]. This complex can be expressed at the cell membrane and presented to other cell types expressing the IL-2R/IL-15R beta-chain and γ c, termed trans presentation (reviewed in [13,14]). Thus, the biological activity of IL-15 differs from traditional secreted cytokines, possibly explaining the difficulty of quantifying IL-15 levels in biological fluids [2].

Initial experiments using recombinant IL-15 protein suggested that IL-15 was an anabolic factor for skeletal muscle. Myotubes of mouse, bovine, and human origin incubated in IL-15 displayed a hypertrophic morphology as well as an increase in myosin heavy chain protein accumulation in culture [15,16]. Therapeutic effects of IL-15 in vivo were demonstrated in diaphragm muscle from young mdx mice (8 weeks), following systemic elevation for 28 days. Muscle cross sectional area (CSA) and specific force ($Po \cdot CSA^{-1}$) were greater in the diaphragm muscles of mdx mice treated with IL-15, compared to control mdx mice [17]. Additional roles of IL-15 in skeletal muscle in vivo include antagonizing protein degradation and inhibiting myonuclear apoptosis in rodent models of cancer [18,19]. In addition to skeletal muscle, IL-15 has potential roles in bone remodeling. IL-15 protein is contained in the synovial fluid of patients affected with rheumatoid arthritis [20] and can stimulate osteoclastogenesis, thus contributing to bone resorption [21].

More recently, a muscle-to-adipose tissue signaling pathway has been proposed whereby IL-15 derived from skeletal muscle could have effects on adipose cells [22]. Administration of IL-15 to Wistar rats for 7-days decreased white adipose tissue mass and circulating triacylglycerols [23]. Interleukin-15 also enhanced lipolysis of adipocytes in vitro [24]. This response appears to be a direct effect of the cytokine, as incubation of IL-15 in differentiating preadipocytes reduced lipid accumulation in vitro [25] and reduced incorporation of a ¹⁴C-triolein in adipose tissue in vivo [26]. In addition to influencing lipid metabolism, IL-15 has been shown to increase glucose uptake and utilization by skeletal muscle [27]. Collectively, IL-15 may have significant influence on both lipid and glucose metabolism, and may therefore prove useful as an antidiabetogenic agent, as previously suggested [27]. These influences may also extend to contributing mechanisms of the metabolic syndrome, as alterations in cholesterol, triglycerides and glucose tolerance are factors in the clinical definition of metabolic syndrome presented by the International Diabetes Federation [28]. Additional factors included in the metabolic syndrome classification include: central obesity, hypertension, and presence of type II diabetes.

Numerous single nucleotide polymorphisms (SNPs) have been identified in both the human IL-15 gene and IL-15R α gene and studied for associations with symptoms of asthma, liver graft rejection, allergies, CD4⁺ T cell activity, skeletal muscle responses to resistance exercise, and obesity [29-36]. A study by Riechman et. al. [35] demonstrated associations between SNPs in the human IL-15R α gene and adaptations to a resistance training program. In that study, 153 Caucasian males and females performed a 10-week total body resistance training program. Strength was measured using a 1 repetition maximum (1RM) protocol and hypertrophy was assessed by circumference measures of the upper arm and thigh. Following training, two SNPs in the IL-15R α gene were associated with increases in muscle hypertrophy. However, this study found no associations with strength measures and muscle quality measures (one-repetition maximum/arm/leg circumference⁻¹) following training. In the present study, we wanted to further examine these SNPs in a larger sample size of subjects performing a unilateral RT program.

Based on the demonstrated roles of the IL-15/IL-15R system presented above, we examined SNPs in the human IL-15 and IL-muscle, and bone phenotypes at baseline and in response to a 12-week unilateral RT program, as well as with predictors of the metabolic syndrome. A primary aim of this study was to identify whether associations existed between these SNPs and predictors of the metabolic syndrome. A secondary aim of this study was to validate a previous report in which associations were observed between SNPs in the IL-15R α gene and skeletal muscle responses to RT, and extend those associations to SNPs in the IL-15 gene. We hypothesized that IL-15 and IL-15R α genotypes would be associated with predictors of the metabolic syndrome as well as skeletal muscle and bone phenotypes examined at baseline and in response to RT.

2. Materials and Methods

2.1. Study Overview

This study is part of a multi-center investigation designed to uncover novel SNPs with associations to human muscle size and strength (Functional SNPs Associated with Human Muscle Size and Strength or FAMuSS). Detailed methodology of the FAMuSS study has been presented previously [37-40]. Briefly, subjects were recruited to complete a 12-week progressive RT program aimed at increasing the strength and size of the elbow flexor and extensor muscles of the non-dominant arm only. Isometric and dynamic strength of the elbow flexors was determined before and after RT. Muscle size of the upper arm was measured by two methods: with a tape during muscle contraction and by magnetic resonance imaging (MRI) to determine muscle volume. DNA samples, obtained from subjects' white blood cells, were collected prior to training and used to perform SNP analyses. Respective institutional review boards at each site approved the study protocol and all subjects read and signed an informed consent document.

2.2. Subject Population

Caucasian men and women were included in this study if they were between the ages of 18 and 40 years, and generally healthy. For statistical analyses, subjects were grouped into cohorts based on the most current data available. Thus, changes in strength were analyzed in a cohort of 748 subjects (448 females, 300 males), changes in muscle volume were analyzed in a cohort of 722 subjects (432 females, 290 males), and serum measures were analyzed in a cohort of 544 subjects (342 females, 202 males). Additional exclusion criteria included the following: use of medications that affect skeletal muscle growth; restriction of activity for medical reasons; chronic medical conditions; metal implants that would prohibit MRI testing; resistance training or a job requiring repetitive use of the arms for at least 12 months prior; use of

supplements designed to improve muscle size or strength (creatine, protein, etc); habitual alcohol use; pregnancy or use of the birth control shot.

2.3. Anthropometric measurements

Bodyweight was measured prior to and following 12-weeks of RT. Stature was measured with a standard tape and BMI was calculated based on pre- and post-training values ($\text{kg}\cdot\text{m}^{-2}$). The waist circumference was measured with a standard tape at the narrowest point between the umbilicus and the xiphoid process of the sternum. Subcutaneous fat was measured using standard skinfold techniques over the biceps brachii and triceps brachii. Subjects' resting heart rate and blood pressure were measured at baseline following 5-minutes in a seated position.

2.4. Maximal Isometric Contraction Test

Maximal voluntary contraction (MVC) of the elbow flexors was tested prior to and following 12-weeks of RT on a specially designed and modified preacher bench using a strain gauge attached to a strength evaluation system (Model 32628CTL, Lafayette Instrument Company, Lafayette, IN). Baseline isometric testing was performed over three days, separated by no more than 48h, and over two days at the completion of training. The average of the second and third testing days was used as the baseline criterion measurement. Subjects' were positioned with their arms at a 90° angle and with the medial epicondyle in line with the axis of rotation of the bench. Three tests lasting 3s were performed on each arm, all separated by a 1-minute rest period. The three peak force values were averaged for each testing day. Isometric strength gain was determined by calculating the percent difference between post-training and pre-training maximal values.

2.5. One repetition maximum (1RM) testing

A 1RM protocol modified from Baechle [41] was used to measure dynamic strength of the elbow flexors on a standard preacher bench prior to and following 12-weeks of RT. Two warm-up sets were completed at 50% and 75% of the predicted 1RM for 8 repetitions and 5 repetitions, respectively. Single attempts were performed until one single repetition with full-range of motion was completed. Dynamic strength gain was determined by calculating the percent difference between post-training and pre-training 1RM values.

2.6. Volumetric measurements

Magnetic resonance imaging was used to assess changes in whole muscle, subcutaneous fat, and total and cortical bone volume of each arm. Pre-training MRI scans were performed before or 48h after any isometric or 1RM testing and post-training MRIs were performed 48–96h after the last training session in order to avoid any temporary water shifts that would skew measurements while also avoiding detraining. Prior to the MRI, the maximal circumference of the upper arm was assessed with the subjects' arms abducted 90° at the shoulder, 90° at the elbow, and maximally contracted. The point of maximal circumference was marked with a radiographic bead (Beekley Spots, Beekley Corp., Bristol, CT).

Rapidia®, a PC-based, three-dimensional, interactive system for viewing images from CT and MRI scans, was used to analyze all volumetric measurements. To ensure accurate and reliable measurements, six slices from each image were analyzed using the metaphyseal-diaphyseal junction landmark, making sure the same regions were measured from pre-and post- images. Bone, subcutaneous fat and muscle were isolated using image signal intensity differences between tissues, and once the region of interest was segmented, total volume was taken for the six evaluated slices. Repeatability and reliability of Rapidia volume measurements were verified using a phantom of known volume.

2.7. Muscle Quality Determination

Muscle quality values were calculated using the volumetric data obtained from the MRI scans and Rapidia software, and was determined for both MVC and 1RM values at baseline and post-training using the following calculation: [force (strength)/muscle volume] [35,42].

2.8. Serum Measures

Subjects reported to the testing laboratories for blood collection during the morning hours after an overnight fast of at least 12 hours. Blood samples were collected at baseline into tubes containing no additive (Vacutainer, BD Biosciences, Franklin Lakes, NJ). Serum was separated by spinning the tubes in a refrigerated centrifuge at 4°C and a speed of 2000rpm for 5-minutes. Serum was aliquoted into 1-ml cryovials and stored at -80°C. Serum samples were analyzed by Quest Diagnostics (Chantilly, VA, 20153) for the following: fasting glucose (Test Code: 483X), coronary risk profile (Test Code: 17512X) that consisted of total cholesterol and high density lipoprotein (HDL), and fasting insulin (Test Code: 561X). Low density lipoprotein (LDL) was determined by subtracting HDL values from the total cholesterol values. The homeostasis model assessment (HOMA) values were obtained from the following calculation: $[(\text{insulin} \times \text{fasting glucose})/22.5]$ [43]. These variables have been reported previously in this subject population [44].

2.9. Exercise training program

A monitored, progressive 12-week unilateral upper arm RT program was initiated following baseline anthropometric and strength measurements. The 1RM values were used to assign appropriate weights in the following training progression: weeks 1–4 – 3 sets @ 12RM, weeks 5–9 – 3 sets @ 8RM, and weeks 10–12 – 3 sets @ 6RM. The order of exercises was as follows: preacher curl, seated overhead triceps extension, concentration curl, triceps kickback, and standing biceps curl. Each repetition was performed through a full range of motion, using dumbbells (Power Blocks, Intellbell Inc., Owatonna, MN). The subject trained their non-dominant arm twice per week with a 24–48h rest period between training sessions. Although the primary focus of testing was the biceps brachii, the triceps brachii were trained to avoid creating a muscle imbalance across the joint.

2.10. Genotyping

Blood samples were obtained from all individuals in ethylenediaminetetraacetic (EDTA) anti-coagulant, sent to the coordinating site in Washington DC without subject identification, and DNA isolated using Qiagen kits. Genotypes for these SNPs were obtained with the use of a TaqMan allelic discrimination assay that employs the 5' nuclease activity of Taq polymerase to detect a fluorescent reporter signal generated during polymerase chain reactions (PCR). The PCR reactions for the each SNP contained 20 ng DNA, 900 nM primers, 200 nM probes, and TaqMan® Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems, Foster City, CA, USA) in a final volume of 15 µl. PCR was performed on a MJ Research Tetrad® thermal cycler (Waltham, MA, USA). The primers and probes for each SNP are located in Table 1. The PCR profile was 10 min at 95 °C (denaturation), 44 cycles of 15 s at 92 °C and 1 min at an annealing temperature of 60 °C. All PCR reactions were analyzed using an ABI 7900 real-time PCR system (Foster City, CA, USA).

2.11. SNP Identification

The genomic location of each SNP analyzed in this paper is presented in Figure 1. A review of the literature was performed to identify SNPs in the IL-15 and IL-15Ra genes that could potentially affect muscle phenotypes, metabolic syndrome predictors, and/or IL-15 expression. SNPs in both coding regions as well as regulatory regions (i.e. 5'UTR, 3'UTR) of the IL-15Ra gene with previously established associations with muscle phenotypes were used in

our analyses [35]. To date, there have been no SNPs described in coding regions that alter IL-15 structure or expression [45]. Therefore, we examined SNPs in the 5' and 3' UTR regulatory regions of the IL-15 gene which had demonstrated associations with expression levels and arthritis [45].

2.11. Statistical analyses

Hardy-Weinberg equilibrium was determined for both IL-15 and IL-15R α SNPs using a χ^2 test to compare the observed genotype frequencies to those expected under Hardy-Weinberg equilibrium. All SNPs were within Hardy-Weinberg equilibrium ($p > 0.05$). Twenty-two size/strength phenotypes and ten phenotypes related to metabolic syndrome were analyzed as continuous quantitative traits. These included: size/strength (baseline measures of whole arm, whole muscle, total bone, cortical bone and subcutaneous fat volume, widest upper arm circumference, 1-RM strength, MVC strength, muscle quality using 1RM, and muscle quality using MVC; difference in whole arm, whole muscle, total bone, cortical bone and subcutaneous fat volume, widest upper arm circumference, 1-RM strength and MVC from baseline to post exercise, the % change in 1-RM strength and MVC from baseline to post-exercise; and muscle quality using 1RM and MVC measures at post-training); metabolic syndrome (BMI, fasting glucose, triglycerides, cholesterol, LDL, HDL, fasting insulin, mean blood pressure, HOMA, and presence of metabolic syndrome). Normality of each quantitative trait was confirmed using the Shapiro-Wilk normality test. Bivariate analyses of each quantitative measurement showed several significant correlations with both age and baseline body mass; therefore, associations between SNPs and size/strength phenotypes were assessed using analysis of covariance methods. Due to large gender differences in baseline values and the response to training, all analyses were performed separately for males and females, and all ANCOVAs included age and/or baseline body mass as covariates.

All significant associations from the main analysis of covariance model were subjected to pairwise statistical tests between each of the three genotype groups for each SNP. Linear tests were performed between each of the genotype groups to determine which genotype groups were significantly different from one another. The resulting p-values from these linear tests were adjusted for multiple comparisons using the Sidak post-hoc multiple comparison test. Linear regression analysis, including likelihood ratio tests between full and constrained models, was performed to estimate the proportion of variance in muscle size/strength measurements attributable to IL-15 and/or IL-15R α genotypes. The presence of metabolic syndrome was tested with each IL-15 and IL-15R α SNP using a logistic regression model adjusted for age and baseline body mass. Dominant genetic models (homozygous wildtype individuals versus heterozygous/homozygous mutant individuals) were used for all analyses, based on the observation that the adjusted means for homozygous mutant and heterozygous individuals were similar and further from the adjusted means for the homozygous wildtype individuals. This indicated to us an overall dominant effect. As a comparison, data were also analyzed using an additive model (homozygous wildtype versus heterozygous versus homozygous mutant) as well as a recessive model (heterozygous/homozygous wildtype versus homozygous mutant) and are presented as supplementary data (see Supplementary Tables 1 and 2).

2.12. Linkage Disequilibrium

Pairwise tests for linkage disequilibrium (LD) were performed for the two IL-15 SNPs and three IL-15R α SNPs using Lewontin's D' and R^2 calculations for each pairwise combination.

3. Results

3.1. Subject Characteristics

The subject characteristics for female and male subjects in three sub-cohorts (strength, volumetric, serum) are presented in Table 2 and the genotype distributions of each sub-cohort are presented in Table 3. Although Caucasians were not the only ethnic group in the study, all analyses were performed in Caucasians which accounted for over 75% of the study population. Due to some missing genotypes, demographic characteristics were calculated for each sub-cohort using the largest possible sample size. No differences were observed in the average age of subjects when comparing females and males. For each sub-cohort, males had a greater baseline body mass, height, and BMI when compared to female subjects (Table 2).

3.2. Linkage Disequilibrium for IL-15 and IL-15R α SNPs

The two SNPs in the IL-15 gene analyzed in this study were in close LD, with a D' value of 0.84. We also observed strong LD values for the SNPs in the IL-15R α gene, as follows: rs3136618 and rs2228059 – D'=0.93; rs2296135 and rs2228059 – D'=0.79; rs2296135 and rs3136618 – D'=0.80.

3.3. IL-15 and IL-15R α SNPs and Associations with Predictors of the Metabolic Syndrome

Significant associations with SNPs in the regulatory elements (1st intron, 3'UTR) of the IL-15 gene were observed with predictors of the metabolic syndrome (Figure 2). The presence of the T-allele in the 1st intron of the rs1589241 SNP was associated with higher values for serum cholesterol (Figure 2A) and LDL (Figure 2B) in female subjects and BMI (Figure 2C) and HOMA (Figure 2D) in male subjects. Additionally, the presence of the T-allele in the 3'UTR of the rs1057972 IL-15 SNP was associated with lesser values for BMI (Figure 2E) and fasting glucose (p=0.03) in males (Figure 2F). When analyzing SNPs in the IL-15R α gene, the presence of the C-allele of the 3rd exon in the rs2228059 IL-15R α SNP was associated with lesser values for serum triglycerides in males (Figure 2G).

3.4. IL-15 and IL-15R α SNPs and Associations with Muscle, Strength, and Bone Phenotypes

Significant associations between SNPs in the IL-15R α and IL-15 genes and muscle, strength, and bone phenotypes were observed in our sample population (Figure 3). The presence of the A-allele in the 3rd exon of the rs2228059 IL-15R α SNP was associated with greater whole muscle volume (Figure 3A) but lower muscle quality measured at baseline in males (Figure 3B). The C-allele in the 3'UTR of the rs2296135 IL-15R α SNP was associated with greater improvements in post-training isometric strength in females (Figure 3C). The T-allele of the 3'UTR of the rs1057972 IL-15 SNP was associated with greater improvements in post-training 1RM in males (Figure 3D). Additionally, the A-allele in the 3'UTR of the rs2296135 IL-15R α SNP was associated with a greater baseline total bone volume in females (Figure 3E). The A-allele in the 3rd exon of the rs2228059 IL-15R α SNP was associated with greater baseline cortical bone volumes in females (Figure 3F).

4. Discussion

The aims of this study were two-fold. First, we wanted to examine whether SNPs in the human IL-15 and IL-15R α genes were associated predictors of the metabolic syndrome. Second, we wanted to determine whether SNPs in the human IL-15 and IL-15R α genes were associated with skeletal muscle and bone phenotypes at baseline and in response to RT. Our data demonstrate that two SNPs in the human IL-15 gene are associated with various predictors of the metabolic syndrome as well as with BMI and muscle strength in a gender-specific manner. Baseline demographic data showed significant differences in body mass, height, and BMI when

comparing female and male subjects. Based on this observation, all phenotype and serum markers were analyzed separately in males and females. However, the reasons for the gender-specific associations are not clear and beyond the scope of this paper, although gender-specific associations have been observed in similar SNP association studies [39,46,47]. Additionally, our data revealed few associations of SNPs in the IL-15R α gene with skeletal muscle adaptations to RT. Collectively, these data demonstrate that IL-15/IL-15R α associated signaling may have roles in the etiology of the metabolic syndrome and with skeletal muscle phenotypes.

4.1. IL-15 SNPs and Associations with the Metabolic Syndrome

Multiple definitions of the metabolic syndrome have been proposed, based on measurable markers of adiposity, insulin resistance, circulating glucose, dyslipidemia, hypertension, etc. In general, factors involved in the metabolic syndrome include: centralized adiposity, glucose intolerance, impaired HDL cholesterol levels, and a blood pressure of greater than 140/90 [48]. In this study, the predictors of metabolic syndrome we were able to measure included: BMI, blood pressure, fasting glucose, serum triglycerides, total cholesterol as well as LDL and HDL, fasting insulin, and the homeostasis model of assessment (HOMA) of insulin resistance. We observed various associations of these predictors with two SNPs in the IL-15 gene that were also gender-specific.

These associations are interesting in light of the proposed role of muscle-derived IL-15 on lipolysis and glycogenolysis [22]. Muscle-derived IL-15 may act on adipose tissue to stimulate lipolysis [24]. This role has been demonstrated both in vitro and in vivo when systemic IL-15 levels have been elevated experimentally [25,26]. The dominant model analysis showed that the presence of the T-allele on of the rs1589241 IL-15 SNP was associated with a greater BMI as well as a greater HOMA measure in male subjects. If muscle-derived IL-15 is indeed able to promote lipolysis, it is conceivable that the presence of the T-allele at this site could alter levels of IL-15 and therefore lead to a reduction in lipolysis and, consequently, a larger BMI. Additionally, as a BMI greater than 25 has been used as one of several predictors of the metabolic syndrome [48], it is not surprising that the presence of the T-allele at this site is also associated with greater HOMA values, indicative of an increase in insulin resistance. The presence of the T-allele also was associated with greater serum values for total cholesterol and LDL cholesterol in female subjects.

This proposed role of IL-15 in the etiology of the metabolic syndrome also holds when examining the presence of the A-allele of the rs1057972 IL-15 SNP. In male subjects, the presence of the A-allele was associated with greater fasting glucose values as well as a greater BMI. Again, if we adopt a BMI value of greater than 25 as a predictor of metabolic syndrome, then it is not surprising that those male subjects with the A-allele at this site would have a greater fasting glucose value. These observations, coupled with the proposed role of muscle derived IL-15 in promoting lipolysis and adiponectin secretion from adipose tissue [23,25], suggests this cytokine may have a role in the metabolic syndrome etiology. Although our subjects were not diagnosed with metabolic syndrome, the associations observed in this study support the possibility of developing metabolic syndrome since various predictors were associated with the SNPs examined in our study population.

4.2. IL-15/IL-15R α System in Skeletal Muscle

Previous studies have identified IL-15 as an anabolic cytokine in myogenic cultures. Myotubes of murine [15], bovine [15], and human [16] origin all increase the protein content of myosin heavy chain when IL-15 is included in culture media. This anabolic effect is distinct from the well-associated anabolic effects of insulin-like growth factor-1 (IGF-1) [15]. In vivo, IL-15 has been shown to decrease the rate of protein degradation [18] and the incidence of apoptosis

within skeletal muscles in a rodent model of cancer [19]. Recently, IL-15 mRNA has been shown to be increased in the muscles of aged animals and in muscles exposed to atrophic stimuli, such as hindlimb suspension and unweighting following stretch-induced hypertrophy [49]. Collectively these data demonstrate that IL-15 expression within skeletal muscle is affected by conditions that promote muscle atrophy and that IL-15 may be able to counter muscle loss induced by disease.

A previous report had demonstrated that IL-hypertrophic response of skeletal muscle following RT. The current study however, conflicts with the data of Riechman et al. [35]. Specifically, our data show associations with one SNP in exon 4 of the IL-15R α gene with muscle phenotypes at baseline, but not in response to our RT program. Notable methodological differences existed between the current study and the study by Riechman et al. [35], which may have led to the differing results. Specifically, the study by Riechman et al. [35] used 153 male and female Caucasian subjects who performed a 10-week total body progressive RT program. Muscle adaptations were determined using circumference measures of the upper arm and upper leg musculature to assess changes in muscle size while 1RM was used to determine changes in muscle strength. In the present study, skeletal muscle phenotypes were obtained using quantitative MRI image analyses in a larger sample size of subjects, as opposed to arm and leg circumference measures. We did analyze a subset of circumference measures of the upper arm in our data set in a further attempt to validate the previous study. However, this also led to differing results as the circumference measures in the current study were obtained by measuring a maximally contracted upper arm, while those in the Riechman et al. [35] study were obtained using a relaxed arm.

Our data demonstrated significant associations with baseline upper arm circumference measures and muscle quality. However, there were no associations with upper arm adaptations to the RT program we utilized. A final difference in these two studies was that the RT performed by subjects in the current study was concentrated to the muscles of the subject's non-dominant upper arm (biceps brachii, triceps brachii), using the dominant arm as a non-exercised control. These methods allowed us to minimize any influence of day-to-day activities that could contribute to the increases in arm size we had previously observed [50]. The methodological differences in this study and those of Riechman et al. [35] could explain why we did not observe associations with IL-15R α SNPs and the muscle responses to RT.

4.3. IL-15 SNPs and Bone

It has been previously shown that IL-15 is contained in the synovial fluid of joints in patients with rheumatoid arthritis (RA) [20]. However, a recent study that analyzed 13 SNPs in the IL-15 gene for associations with RA risk found no role for these SNPs in genetic predisposition to RA [45]. It has also been shown that IL-15 protein is measurable within human osteoblast-like cells [51] and that IL-15 has the ability to promote osteoclastogenesis, thus contributing to bone loss [21]. Our data highlight novel associations between SNPs in the IL-15R α gene and both total bone volume and cortical bone volume measured at baseline of our study. These data collectively suggest that IL-15 may have a role in bone formation and/or bone resorption that requires further study.

5. Conclusions

In this study, we sought to identify associations between SNPs in the IL-15 and IL-15R α genes and predictors of the metabolic syndrome as well as muscle and bone phenotypes in response to RT. Our data highlight novel associations between predictors of the metabolic syndrome, specifically, cholesterol, BMI, fasting glucose, and HOMA measures and SNPs in these genes, which were gender-specific. However, we were not able to confirm previously observed associations between SNPs in the IL-15R α gene and muscle responses to RT, although

methodological differences were noted between studies. Our data did demonstrate that SNPs in the IL-15R α gene were associated with skeletal muscle phenotypes at baseline, suggesting a role of the IL-15/IL-15R α pathway in muscle development. Collectively, the IL-15 specific associations with the predictors of the metabolic syndrome and the observed properties of IL-15 on adipose tissue metabolism suggest this cytokine may have a role in the etiology of the metabolic syndrome and in skeletal muscle phenotypes that warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Genomic location of IL-15 and IL-15Rα SNPs

A) The two SNPs in the IL-15 gene analyzed in this study were located within the first intron (rs1589241) and the 3' UTR (rs1057972). B) The three SNPs in the IL-15Rα gene analyzed in this study were located in the 3rd exon (rs2228059), the 4th intron (rs3136618), and the 3' UTR (rs2296135). Black boxes = exons; gray boxes = untranslated regions.

Figure 2. SNP Associations Between IL-15 and IL-15R α Genes and Predictors of the Metabolic Syndrome

A dominant model analysis was used to examine associations between SNPs and various predictors of the metabolic syndrome in male and female subjects. These associations were gender specific and each graph contains the both genders for comparison. Significant associations were observed between rs1589241 and total cholesterol (A) and LDL cholesterol (B) in female subjects, and with BMI (C) and HOMA (D) in male subjects; rs1057972 and fasting glucose (F) and BMI (E) in male subjects; and rs2228059 and serum triglycerides in male subjects (G). $p<0.05^*$.

Figure 3. SNP Associations Between IL-15 and IL-15R Genes and Muscle, Strength, and Bone Phenotypes

A dominant model analysis was used to examine associations between SNPs and measures of muscle volume, strength, and bone volume in male and female subjects. These associations were gender specific and each graph contains the both genders for comparison. Significant associations observed between rs2228059 and baseline whole muscle volume (A) and muscle quality (B) in male subjects; rs2296135 and the post-training difference in isometric strength (C) in females; rs1057972 and the post-training difference in 1RM strength (D) in males; rs2296135 and baseline total bone volume (E) in females; and rs2228059 and baseline cortical bone volume (F) in males. $p < 0.05^*$.

Table 1

TaqMan Primer Sets for IL-15 and IL-15Rα SNPs

Gene	SNP	Forward Primer	Reverse Primer	WT allele probe (5' VIC®)	MT allele probe (5' FAM)
IL15	rs1589241	TCACTGGATTCTCTG CCTTTACTG	GAAATTGACATCAGGTAAGATG AGGAAGA	ATATCTGCTTGCCC CACCT	ATATCTGCTTGCTC CACCT
IL15	rs1057972	TGCAGGTCAACAG CTATGCT	AGTCAATGAGAGCCAGTAGTCA GT	TCCTGCCTGTGTGG AA	CCTGCCAGTGTGGA A
IL15Rα	rs2228059	GCACCCCTCTCTCAG ACAA	GAGGCGGATGCTGTGAGT	CAGCCAAGAACTGG GA	CAGCCAAGACCCTGG GA
IL15Rα	rs3136618	CAGCGACACCACT GGTAAGT	CTGGGGTCACCCCTGATCATA	CCCTTGTCCAGTA TGTT	CCTTTGTCCGGTAT GTT
IL15Rα	rs2296135	AGAGAGCTTTGGG TATGTCACATTTT	AACCCCTGCCCCCGAATCTTC	CATCCTAACTTGA GTTCA	TCATCCTAACTTTC AGTTCA

WT=wild type, MT=mutant type, VIC/FAM = reporter dyes

Table 2 Demographic characteristic of FAMuSS sub-cohorts:

Characteristic	Females		Males		P-value
	N*	Mean ± SD	N*	Mean ± SD	
Strength sub-cohort					
Age (years)	448	23.11 ± 5.51	300	23.67 ± 5.51	NS
Baseline body mass (kg)	448	65.32 ± 12.79	300	79.98 ± 16.11	<0.0001
Baseline height (cm)	448	165.26 ± 6.60	300	178.01 ± 6.84	<0.0001
Baseline BMI	448	23.91 ± 4.54	300	25.21 ± 4.76	0.0001
Volumetric sub-cohort					
Age (years)	432	23.08 ± 5.49	290	23.67 ± 5.50	NS
Baseline body mass (kg)	432	65.56 ± 12.87	290	79.76 ± 16.13	<0.0001
Baseline height (cm)	432	165.27 ± 6.57	290	177.97 ± 6.89	<0.0001
Baseline BMI	432	23.99 ± 4.56	290	25.15 ± 4.75	0.0042
Serum sub-cohort					
Age (years)	342	23.56 ± 5.59	202	24.24 ± 5.88	NS
Baseline body mass (kg)	342	65.09 ± 12.81	202	81.08 ± 16.92	<0.0001
Baseline height (cm)	342	165.13 ± 6.76	202	178.04 ± 6.74	<0.0001
Baseline BMI	342	23.88 ± 4.66	202	25.55 ± 5.05	0.0001

Table 3

Genotype distributions of FMS sub-cohorts:

SNP	Females			Males		
	Homozygous wildtype	Heterozygous	Homozygous mutant	Homozygous wildtype	Heterozygous	Homozygous mutant
Strength sub-cohort						
IL15 (rs1057972)	127 (28.7%, AA)	225 (50.9%, AT)	90 (20.4%, TT)	71 (24.1%, AA)	175 (59.3%, AT)	49 (16.6%, TT)
IL15 (rs1589241)	235 (54.4%, CC)	164 (38.0%, CT)	33 (7.6%, TT)	149 (51.4%, CC)	117 (40.3%, CT)	24 (8.3%, TT)
IL15RA (rs3136618)	110 (26.1%, GG)	219 (52.0%, GA)	92 (21.9%, AA)	68 (25.4%, GG)	140 (52.2%, GA)	60 (22.4%, AA)
IL15RA (rs2228059)	114 (27.8%, AA)	207 (50.5%, AC)	89 (21.7%, CC)	72 (27.4%, AA)	129 (49.1%, AC)	62 (23.6%, CC)
IL15RA (rs2296135)	118 (26.3%, CC)	227 (50.7%, CA)	103 (23.0%, AA)	66 (22.0%, CC)	173 (57.7%, CA)	61 (20.3%, AA)
Volumetric sub-cohort						
IL15 (rs1057972)	99 (31.0%, AA)	159 (49.8%, AT)	61 (19.1%, TT)	47 (24.1%, AA)	110 (56.4%, AT)	38 (19.5%, TT)
IL15 (rs1589241)	163 (52.4%, CC)	120 (38.6%, CT)	28 (9.0%, TT)	107 (55.7%, CC)	72 (37.5%, CT)	13 (6.8%, TT)
IL15RA (rs3136618)	78 (25.8%, GG)	164 (54.3%, GA)	60 (19.9%, AA)	47 (27.3%, GG)	89 (51.7%, GA)	36 (21.0%, AA)
IL15RA (rs2228059)	80 (27.5%, AA)	153 (52.6%, AC)	58 (19.9%, CC)	50 (29.2%, AA)	87 (50.9%, AC)	34 (19.9%, CC)
IL15RA (rs2296135)	82 (25.5%, CC)	168 (52.2%, CA)	72 (22.4%, AA)	45 (22.5%, CC)	115 (57.5%, CA)	40 (20.0%, AA)
Serum sub-cohort						
IL15 (rs1057972)	99 (28.0%, AA)	183 (51.7%, AT)	72 (20.3%, TT)	51 (23.8%, AA)	128 (59.8%, AT)	35 (16.4%, TT)
IL15 (rs1589241)	183 (53.2%, CC)	134 (39.0%, CT)	27 (7.8%, TT)	107 (51.2%, CC)	85 (40.7%, CT)	17 (8.1%, TT)
IL15RA (rs3136618)	89 (26.0%, GG)	178 (52.1%, GA)	75 (21.9%, AA)	52 (25.7%, GG)	106 (52.5%, GA)	44 (21.9%, AA)
IL15RA (rs2228059)	93 (28.0%, AA)	168 (50.6%, AC)	71 (21.4%, CC)	55 (27.8%, AA)	96 (48.9%, AC)	47 (23.7%, CC)
IL15RA (rs2296135)	86 (24.0%, CC)	184 (51.4%, CA)	88 (24.6%, AA)	48 (22.3%, CC)	122 (56.7%, CA)	45 (20.9%, AA)