Muscle-specific deletion of exons 2 and 3 of the *IL15RA* gene in mice: effects on contractile properties of fast and slow muscles

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1 Division of Exercise Physiology, West Virginia University, Morgantown, West Virginia; 2 Center for Cardiovascular and Respiratory Sciences, West Virginia University, Morgantown, West Virginia; 3 West Virginia Clinical and Translational Science Institute, West Virginia University, Morgantown, West Virginia; 4 Department of Physiology and Pharmacology, West Virginia University, Morgantown, West Virginia; 5 Geriatric Research, Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, Washington; 6 Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, Washington; and 7 Department of Medicine, University of California, San Francisco, San Francisco, California

Submitted 4 August 2014; accepted in final form 3 December 2014

*O’Connell G, Guo G, Stricker J, Quinn LS, Ma A, Pistilli EE.* Muscle-specific deletion of exons 2 and 3 of the *IL15RA* gene in mice: effects on contractile properties of fast and slow muscles. *J Appl Physiol* 118: 437–448, 2015. First published December 11, 2014; doi:10.1152/japplphysiol.00704.2014.—Interleukin-15 (IL-15) is a putative myokine hypothesized to induce an oxidative skeletal muscle phenotype. The specific IL-15 receptor alpha subunit (IL-15Rα) has also been implicated in specifying this contractile phenotype. The purposes of this study were to determine the muscle-specific effects of IL-15Rα functional deficiency on skeletal muscle isometric contractile properties, fatigue characteristics, spontaneous cage activity, and circulating IL-15 levels in male and female mice. Muscle creatine kinase (MCK)-driven IL-15Rα knockout mice (mIL15rαfl/fl/Cre) were generated using the Cre-loxP system. We tested the hypothesis that IL-15Rα functional deficiency in skeletal muscle would increase resistance to contraction-induced fatigue, cage activity, and circulating IL-15 levels. There was a significant effect of genotype on the twitch:contraction ratio, such that the twitch:contraction ratio was lower in these muscles compared with muscles from control mice. There was a significant effect of genotype on the fatigue characteristics of skeletal muscle in vitro (31, 35, 37). For example, transgenic mice that secrete large amounts of IL-15 from skeletal muscle have increased exercise endurance in conjunction with greater expression of oxidative genes in muscles (37). These mice are also leaner and are resistant to high-fat diet-induced obesity, suggesting that IL-15 release from skeletal muscle can have endocrine effects, although, sex-specific differences in body composition were observed (40). Somewhat paradoxically, global tissue knockout of the IL-15Rα is associated with greater circulating IL-15 levels and a similar muscle and exercise phenotype as that described in the muscle-specific IL-15-overexpressing mouse (16, 31). In male global tissue IL-15Rα knockout (KO) mice, exercise capacity is greater than in background control mice, and this is accompanied with a more fatigue-resistant oxidative phenotype in glycolytic muscles (31). The greater exercise capacity in this mouse strain was observed in a separate study in female mice, demonstrating similar effects of IL-15Rα deficiency between sexes (16). These studies provide associative data between increased circulating IL-15 and pro-oxidative changes in skel-
et al muscle in two different transgenic mouse models. These pro-oxidative changes may be mediated through the ability of IL-15 to induce the transcription factor peroxisome proliferator-activated receptor delta (PPARδ) and stimulate oxidative remodeling in skeletal muscle (38).

In contrast to cytokines such as IL-6 that are secreted in high amounts from skeletal muscle, IL-15 may not be secreted from tissues in high amounts under normal physiological conditions (29, 30, 48). Secretion, stability, and possibly the bioactivity of IL-15 are regulated by IL-15Rα. IL-15 and IL-15Rα interact intracellularly, and the subsequent formation of the IL-15/IL-15Rα complex may represent the true biological form of the molecule. Binding between IL-15 and IL-15Rα occurs via a sushi domain which is coded for in exon 2 of the IL15RA gene (11). In addition, the linker region of IL-15Rα which is coded for in exon 3 also contributes to IL-15 high-affinity binding mainly by slowing dissociation rates (5). The cytoplasmic portion of IL-15Rα does not transduce any IL-15-mediated signaling (2). Therefore, the role of IL-15Rα is predominantly to bind to IL-15, form the IL-15/IL-15Rα complex, and direct the complex to the appropriate compartment within the cell or for secretion.

The purposes of this study were to determine the muscle-specific effects of IL-15Rα functional deficiency on skeletal muscle isometric contractile properties, fatigue characteristics, spontaneous cage activity, and circulating IL-15 levels in male and female mice. Using the Cre-loxP system under the control of the muscle creatine kinase (MCK) promoter, we selectively deleted exons 2 and 3 of the IL15RA gene in skeletal muscle and cardiac tissue. Targeting exons 2 and 3 via the Cre-loxP system will result in nonfunctional IL-15Rα, incapable of binding IL-15, after recombination. We hypothesized that IL-15Rα functional deficiency in skeletal muscle would increase resistance to contraction-induced fatigue, cage activity, and circulating IL-15 concentrations. Collectively, our data in this novel mouse strain are partially supportive of our prior observations in the general IL-15Rα mouse (31), with sex-specific effects of muscle IL-15Rα deficiency on peripheral muscle contractile properties and fatigue phenotypes, but not on spontaneous cage activity or circulating IL-15 levels.

**MATERIALS AND METHODS**

**Experimental mice.** Mice with loxP sites flanking exons 2 and 3 of the IL15RA gene were generated on a C57BL/6 background as previously described (26). Transgenic mice with the Cre recombinase gene under the control of the muscle creatine kinase promoter (MCK-Cre+) were obtained from Jackson Laboratories (stock number 006475). Global IL-15RαKO mice (stock no. 003723) and B6129 background control mice (stock no. 101045) were obtained from Jackson Laboratories at 8 wk of age and were used between 10 and 12 wk of age. The tissue was digested overnight at 55°C in 100 μl of tail lysis buffer (Allele Biotechnology, San Diego, CA). The following morning, tissues were checked for sufficient digestion and then returned to the heat block for 15 min at 80°C to denature the proteinase K. PCR reactions were performed to determine the genotype of animals using specific primers for IL-15Rα and MCK-Cre (Table 1). Amplification of IL15RA was performed in a reaction consisting of 8.5 μl nuclease-free H2O, 12.5 μl 2X Taq-Pro Red Complete 1.5 mM MgCl2 master mix (Denville Scientific, Metuchen, NJ), 1.0 μl forward primer, 1.0 μl reverse primer, and 2.0 μl of DNA template to make a 25 μl total reaction volume. IL-15Rα PCR products were amplified for 34 cycles using the following thermal cyclers conditions: 1 min at 94°C; 1 min at 61°C; and 2 min at 72°C. Amplification of MCK-Cre was performed in a reaction consisting of 8.1 μl nuclease-free water, 12.5 μl 2X Taq-Pro Red Complete 1.5 mM MgCl2 master mix (Denville Scientific, Metuchen, NJ), 0.6 μl forward primer, 0.6 μl reverse primer, 0.6 μl internal control forward primer, 0.6 μl internal control reverse primer, and 2.0 μl of DNA template to make a 25 μl total reaction volume. MCK-Cre PCR products were amplified for 35 cycles using the following thermal cyclers conditions: 30 s at 94°C; 30 s at 64°C; and 30 s at 72°C. Following amplification, each reaction was visualized following gel electrophoresis in 1.5% (wt/vol) agarose gels stained with ethidium bromide.

**Analysis of IL-15Rα and IL-15 DNA and mRNA.** Genomic and transcriptional analysis of IL-15Rα was performed in multiple tissue types in mIl15rafl/Cre+ and mIl15rafl/Cre−, along with the Il15rafl/−, Il15rδfl/−, Il15rδfl/Cre−, and MCK-Cre− mice to confirm skeletal and cardiac muscle-specific deletion of exons 2 and 3 of the IL15RA gene in the experimental mice. PCR experiments were also performed in skeletal muscles from the global IL-15RαKO and B6129 background control mice as a comparison between the two strains of IL-15RαKO mice. Quadriceps muscle, heart, liver, spleen, kidney, lung, and brain were harvested and flash frozen in liquid nitrogen. Genomic DNA was isolated from these tissues using a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) according to manufacturer’s instructions, to verify the genotyping results obtained from tail snips and also to determine that germline Cre-recombination did not occur. Primer sequences and PCR protocols are the same as detailed in the genotyping section.

Total RNA was isolated using Trizol reagent (Life Technologies, Grand Island, NY), as previously described (34). RNA quantity and quality were assessed using a NanoDrop 2000 spectrophotometer (ThermoScientific, Waltham, MA); the 260/280 ratio for all samples used was between 1.8 and 2.1. Two micrograms of total RNA was reverse transcribed to make cDNA using a high-capacity reverse transcription kit according to manufacturer’s instructions (Life Technologies, Grand Island, NY). Primers for various exon spanning regions within the IL15RA gene as well as for the IL15 gene were constructed (Table 1). Amplification was performed in a reaction consisting of 8.5 μl nuclease-free H2O, 12.5 μl 2X Taq-Pro Red Complete 1.5 mM MgCl2 master mix (Denville Scientific, Metuchen, NJ), 1.0 μl forward primer, 1.0 μl reverse primer, and 2 μl of DNA template to make a 25 μl total reaction volume. Primer pairs for IL-15 were coamplified with primer pairs for 18S (Ambion, Austin, TX).

The number of PCR cycles was determined in preliminary experiments to ensure analyses were done in the linear range of amplification.
Table 1. PCR primers

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<th>Primer No.</th>
<th>Target exons</th>
<th>Sequence</th>
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<th>Product, bp</th>
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<th>Tm, °C</th>
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<td>520, 404</td>
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<td>55°C</td>
<td>72°C</td>
<td>40</td>
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<tr>
<td></td>
<td></td>
<td>R: 5′-TGGAGGTCATCCATCTTCTGTTTC</td>
<td></td>
<td></td>
<td>30 s</td>
<td>30 s</td>
<td>45 s</td>
<td></td>
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<tr>
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<td>72°C</td>
<td>40</td>
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<td>370, 271, 172</td>
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<td>55°C</td>
<td>72°C</td>
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<td>55°C</td>
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<td>72°C</td>
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<td>Cre Del</td>
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<td>NC_0000068.7</td>
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<td>61°C</td>
<td>72°C</td>
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<td>R: 5′-GGAGCTCAGCAAAAGAAAGTTGG</td>
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<td>60 s</td>
<td>60 s</td>
<td>120 s</td>
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<tr>
<td>10</td>
<td>MCK CRE</td>
<td>F: 5′-GTGAAAACAGACACTGTTCTGACTT</td>
<td>—</td>
<td>94°C</td>
<td>64°C</td>
<td>72°C</td>
<td>35</td>
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<tr>
<td></td>
<td>Int Con</td>
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<td>NC_000080.6</td>
<td>200</td>
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<td>64°C</td>
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F, forward; R, reverse; Tm, melting temperature; Tm, annealing temperature; Tm, extension temperature.

Ex vivo assessment of skeletal muscle contractile properties. Muscle contractile properties were examined in the fast extensor digitorum longus (EDL) and slow soleus muscles of experimental mice when they were between the ages of 10 and 12 wk (i.e., adult). Due to the length of time required to perform contractile measures on both EDL and both soleus muscles, only one mouse was tested per day. To account for day-to-day variability, all contractile experiments were performed between the hours of 8 am and 12 pm. Mice were deeply anesthetized by breathing 4% isoflurane delivered through a nose cone at a flow rate of 1 l/min. Muscles were removed with both proximal and distal tendons intact, and nylon sutures were attached to the tendons. Muscles were transferred to an oxygenated tissue bath that contained Ringer solution (100 mM NaCl, 4.7 mM KCl, 3.4 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM HEPES, and 5.5 mM d-glucose) maintained at 22°C. Ex vivo muscle stimulation was performed using a commercially available muscle physiology system (Aurora Scientific, Ontario, CA). Muscle length was adjusted to obtain the maximal twitch response (i.e., Lo). Three twitch contractions and three tetanic contractions were performed all separated by 2 min. Parameters analyzed from isometric contractions included peak isometric twitch force (Pt), time to peak twitch tension (TPT), half-relaxation time of twitch contraction (½ RT), and peak isometric tetanic force (PTo). Isometric tetanic contractions were stimulated in muscles at a stimulation frequency of 120 Hz for EDL muscles and 80 Hz for soleus muscles, a stimulation current of 20 V, and lasting 500 ms. Following isometric contractions, the muscles remained in the oxygenated tissue bath for 5 min prior to the repeated stimulation fatigue protocol. Muscle fatigue was analyzed using a repeated stimulation protocol lasting 6 min and consisting of repeated 40-Hz tetanic trains that occurred once every second and lasted 330 ms (7, 31). The fatigue index was calculated as the difference in force from the first contraction and every subsequent contraction. Fatigue curves were plotted using absolute forces produced during the repeated stimulation protocol as well as using the fatigue index values. Muscle cross-sectional area (CSA) was calculated by dividing the muscle mass by the product of the muscle density coefficient (1.06 g/cm3), muscle optimal length (Lo), and the fiber length coefficient (EDL = 0.45, soleus = 0.69). This whole muscle CSA value was used to calculate specific force (i.e., absolute force in mN/muscle CSA) (6, 23).

Mitochondrial DNA content. Total DNA (genomic and mitochondrial) was extracted from tibialis anterior (TA) muscles from male and female mice using a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) and quantified using a Nano-Drop spectrophotometer (Thermo-
was rejected when time. Data are presented as means of ANOVA was used to determine the effects of force loss over CT calculation (21). 

Independent variables of sex and genotype, as well as the statistical Bonferroni post hoc test, was used to determine the effects of the GraphPad 5.0 statistical software package. A two-way ANOVA, with Il15rafl/fl/Cre Il15rafl/fl/Cre mice were bred with MCK-Cre mice to generate Il15rafl/fl/Cre mice. Heterozygous Il15rafl/fl/Cre mice were bred to obtain homozygous Il15rafl/fl/Cre mice, 610 bp and 468 bp; wild-type mice, 468 bp; Il15rafl/fl/Cre+ mice, 392 bp (Fig. 1B). In addition, analysis of DNA isolated from multiple organs verified that the 392-bp band obtained following Cre-mediated recombination of the IL15RA gene was present only in skeletal and cardiac muscle samples of Il15rafl/fl/Cre+ mice (Fig. 1C). Total RNA was isolated from the same tissues, and PCR amplification of a sequence

RESULTS

Generation of Il15rafl/fl/Cre+ mice. A three-step breeding process using Il15rafl/fl homoygous mice and MCK-Cre hemizygous mice led to the generation of muscle-specific Il15rafl/fl/Cre+ mice (Fig. 1A). These mice were viable and no obvious phenotypic differences were observed during the first 3 mo after they were born. Analysis of DNA was used to verify the genotype of mice, based on the expected size of the PCR product following amplification of the IL15RA gene. PCR amplification produced the following band sizes: Il15rafl/fl homozygous mice, 610 bp; Il15rafl/fl heterozygous mice, 610 bp and 468 bp; wild-type mice, 468 bp; Il15rafl/fl/Cre+ mice, 392 bp (Fig. 1B). In addition, analysis of DNA isolated from multiple organs verified that the 392-bp band obtained following Cre-mediated recombination of the IL15RA gene was present only in skeletal and cardiac muscle samples of Il15rafl/fl/Cre+ mice (Fig. 1C). Total RNA was isolated from the same tissues, and PCR amplification of a sequence

Statistical analysis. All data were quantified and analyzed using the GraphPad 5.0 statistical software package. A two-way ANOVA, with Bonferroni post hoc test, was used to determine the effects of the independent variables of sex and genotype, as well as the statistical interaction of the two factors. For fatigue index analysis, a two-way ANOVA was used to determine the effects of force loss over time. Data are presented as means ± SD, and the null hypothesis was rejected when P < 0.05. For contractile experiments and muscle wet weights, sample sizes were 14 male Il15rafl/fl/Cre+ mice, 8 male Il15rafl/fl/Cre−, 10 female Il15rafl/fl/Cre+, and 13 female Il15rafl/fl/Cre−. For transcriptional analysis, sample sizes were 6 male Il15rafl/fl/Cre+, 6 male Il15rafl/fl/Cre−, 6 female Il15rafl/fl/Cre+, and 6 female Il15rafl/fl/Cre− mice. For ELISA experiments, sample sizes were 7 male Il15rafl/fl/Cre+, 10 male Il15rafl/fl/Cre−, 7 female Il15rafl/fl/Cre+, and 10 female Il15rafl/fl/Cre− mice.

Fig. 1. Generation and characterization of muscle-specific Il15rafl/fl/Cre+ mice. A: a 3-step breeding process was utilized to generate muscle-specific Il15rafl/fl/Cre+ mice. Heterozygous Il15rafl/fl mice were bred to obtain homozygous Il15rafl/fl mice at an expected frequency of 25%. These homozygous Il15rafl/fl mice were bred with MCK-Cre mice to generate Il15rafl/fl/Cre− mice. Il15rafl/fl/Cre+ mice were bred to homozygous Il15rafl/fl mice to generate muscle-specific Il15rafl/fl/Cre+ mice as well as littermate control Il15rafl/fl/Cre− mice. B: these different mouse strains were distinguishable based on the expected size of the IL15RA PCR product, as follows: homozygous Il15rafl/fl mice = 610 bp; heterozygous Il15rafl/fl mice = 610 bp and 468 bp; wild-type mice = 468 bp; Il15rafl/fl/Cre+ mice = 392 bp. C: DNA isolated from multiple organs revealed the presence of the 392-bp PCR product resulting from MCK-Cre mediated recombination, in skeletal and cardiac muscle of Il15rafl/fl/Cre+ mice (outlined in white box) and not in Il15rafl/fl/Cre− littermate control mice. D: semiquantitative PCR revealed that MCK-Cre-mediated recombination resulted in the loss of exons 2 and 3 in the IL15RA gene in skeletal and cardiac muscle of Il15rafl/fl/Cre+ mice (outlined in white box) and not in Il15rafl/fl/Cre− littermate control mice.

J Appl Physiol • doi:10.1152/japplphysiol.00704.2014 • www.jappl.org
within exons 2 and 3 verified the loss of IL15RA mRNA from within these specific exons occurred only in skeletal and cardiac muscle of mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice (Fig. 1D).

**Body and skeletal muscle weights of mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice.** The body weight and skeletal muscle weights for male and female mIl15ra<sup>fl/fl</sup>/Cre<sup>+</sup> and mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice are presented in Table 2. At 10–12 wk of age, there was no effect of genotype on the body weight of experimental mice, although male mice weighed more than female mice. There were significant effects of genotype for the wet weights of skeletal muscles composed of a high percentage of type II muscle fibers, such that muscle weights of the EDL, tibialis anterior, and quadriceps from mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice weighed less than muscles from mIl15ra<sup>fl/fl</sup>/Cre<sup>+</sup> mice. In contrast, there were no significant effects of genotype for the wet weights of skeletal muscles composed of a greater percentage of type I muscle fibers, including the soleus muscle and gastrocnemius muscle as well as the heart. There was a significant effect of sex for all skeletal muscles and the heart in the experimental mice, such that muscles and hearts from male mice weighed more than muscles and organs from female mice.

**IL-15Ra and IL-15 expression.** To determine which exons were present after Cre-loxP mediated recombination, PCR primers were designed to amplify specific exon spanning regions within the IL15RA gene (Table 1). PCR products were amplified from muscles of mIl15ra<sup>fl/fl</sup>/Cre<sup>+</sup> mice and mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> littermate control mice, as well as the global IL-15RaKO mouse and the B6129 background control mouse. The expression patterns of the specific exons were similar between the mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> littermate control and the B6129 background control mouse, as well as the mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mouse and the global IL-15RaKO mouse, demonstrating similar molecular composition of the IL15RA gene in the muscles of these two genetically modified mouse models (Fig. 2, A and B). In addition, these PCR results demonstrated that exons 1–9 are present in both control strains of mice, while only exons 4–9 are present in both knockout strains of mice. Amplification of a region corresponding to exons 2–3 produced an expected band of 280 bp in both control strains of mice, with no bands in the knockout strains of mice. These results are expected, as exons 2 and 3 were the exons targeted for deletion in both strains of knockout mice. There were no differences in IL-15 mRNA expression in skeletal muscles from mIl15ra<sup>fl/fl</sup>/Cre<sup>+</sup> mice compared with mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice (Fig. 2C). In contrast to our hypothesis, IL-15 protein measured in the serum of mIl15ra<sup>fl/fl</sup>/Cre<sup>+</sup> mice was less than mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> littermate control mice. Female mice had greater circulating levels of IL-15 than male mice (Fig. 2D). These data demonstrate that gene expression patterns for IL15RA are similar in the global IL-15RaKO mouse and the mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup>, as well as the B6129 background control mouse and the mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mouse. In contrast to the global IL-15RaKO mouse (16), IL-15 mRNA expression was not different in muscles of mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice and circulating IL-15 levels were significantly lower compared with mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> littermate control mice.

**Fatigue properties of isolated skeletal muscles.** Resistance to fatigue was analyzed ex vivo in fast EDL and slow soleus muscles through a repeated contraction protocol in which muscles were stimulated for 330 ms every second for 6 min. Fatigue curves were generated by plotting the loss in absolute force over the duration of the protocol, as well as by calculating the percent difference in force of the initial contraction and every subsequent contraction to obtain the fatigue index. The fatigue curves for fast EDL muscles from female and male mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> and mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice are presented in Fig. 3. The loss in absolute force was significantly different when comparing the fatigue curves from EDL muscles of female mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> and mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> during the early phase of the fatigue protocol (genotype: P < 0.0001; Fig. 3A). When the fatigue index curve was generated, the fatigue index of the EDL muscle from female mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice was significantly different from the fatigue curve of EDL muscles from mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice specifically during the early phase of the fatigue protocol; the curves were inseparable after 80 contractions (genotype: P = 0.0013; Fig. 3B). There were minimal differences in the absolute loss of force or the fatigue index in the EDL muscles of male mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> and mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice (Fig. 3, C and D). In the soleus muscles, there were no significant differences in fatigue properties in female mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> and mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice (Fig. 4, A and B). There was a significant effect of genotype on absolute force output during the fatigue protocol when comparing male mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> and mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice; this effect was minimized when calculating the fatigue index (Fig. 4, C and D). The fatigue data in isolated EDL muscles from female mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> and mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice in this study are consistent with a direct effect of IL-15Ra deficiency on skeletal muscle fatigue. However, these changes in fatigue resistance in muscles of mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> were not associated with an increase in voluntary exercise in mIl15ra<sup>fl/fl</sup>/Cre<sup>-</sup> mice, as
there were no significant differences in wheel running performance of these mice compared with controls (Fig. 3E).

Mitochondrial DNA content. Mitochondrial DNA content was used as a surrogate measure of mitochondrial density and determined by utilizing the CT values for the mitochondrial genome-specific COXII gene and the nuclear genome-specific 18S gene in TA muscles from male and female mice to calculate fold change. In muscles from female mIl15rafl/fl/Cre−/H11001 mice, there was a significant twofold difference in COXII levels, suggesting a greater amount of mitochondrial DNA in the muscles of female mIl15rafl/fl/Cre−/H11001 mice compared with control. There were no differences in the CT values for these markers or the fold change in muscles from male mice (Fig. 5).

Isometric contractile properties. Fast EDL and slow soleus muscles were dissected and transferred to a tissue chamber bath containing oxygenated Ringer’s solution for assessment of isometric contractile properties analyzed by genotype and sex. A full listing of contractile data obtained in these muscles is included in Table 3. Representative twitch force traces of the soleus and EDL muscle are presented in Fig. 6.

In the soleus muscle, there were significant sex-specific effects of genotype on the following isometric contractile properties: absolute twitch contractile force (Fig. 6C); specific twitch force (Fig. 6D); twitch:tetanus ratio (Fig. 6E); and 1/2 RT (Fig. 6F). There were fewer differences observed in the EDL muscle when comparing mIl15rafl/fl/Cre+ and mIl15rafl/fl/Cre− mice.

Fig. 2. IL-15 receptor alpha subunit (IL-15Rα) exon-specific PCR and IL-15 PCR. A: PCR amplification of exon spanning regions of the Il15ra gene was performed in skeletal muscles from the mIl15rafl/fl/Cre+ mouse and mIl15rafl/fl/Cre− littermate control mouse. The expected band sizes for all exon spanning regions were visualized in the muscles from the mIl15rafl/fl/Cre+ control mouse. However, amplification for the exon spanning regions 1–4 and 2–3 did not occur (outlined in white box) in muscles from the mIl15rafl/fl/Cre− mouse. B: PCR amplification of exon spanning regions of the Il15ra gene was performed in skeletal muscles from the global IL-15RαKO mouse and the B6129 control mouse. The expected band sizes for all exon spanning regions were visualized in the muscles from the B6129 control mouse. However, amplification for the exon spanning regions 1–4 and 2–3 did not occur (outlined in white box) in muscles from the global IL-15RαKO mouse. C: Il15 mRNA abundance in skeletal muscles from mIl15rafl/fl/Cre−/H11001 mice was not different compared with muscles from the mIl15rafl/fl/Cre−/H11002 control mouse. D: circulating IL-15 levels were significantly lower in mIl15rafl/fl/Cre+ mice compared with mIl15rafl/fl/Cre− control mice; female mice had greater circulating levels of IL-15 compared with male mice. *Significant effect of genotype. #Significant effect of sex.
There was a significant effect of genotype on the twitch:tetanus ratio (Fig. 6G). The alterations in the twitch:tetanus ratio and in 1/2 RT are consistent with a shift in the oxidative properties of the muscles from \textit{mIl15rafl/fl/Cre} mice.

**DISCUSSION**

The muscle-specific \textit{mIl15rafl/fl/Cre} mouse was generated to determine the direct effects of functional IL-15Rα deficiency in skeletal muscle on contractile properties, fatigue characteristics, spontaneous cage activity, and circulating IL-15 levels. Our laboratory has previously demonstrated that male global IL-15Rα KO mice, first generated in 1998 (22), exhibit greater exercise capacity and fatigue-resistant fast muscles (31). This phenotype was associated with an increase in overall muscle mitochondrial content as well as greater levels of muscle IL-15 mRNA and circulating IL-15 protein levels (16, 31, 33, 38). However, inasmuch as IL-15Rα is expressed in a wide variety of immune and nonlymphoid cells (12), the extent to which the observed exercise and muscle performance phenotype might be due to a direct effect of IL-15Rα deficiency in skeletal muscle or a secondary effect of IL-15Rα deficiency in another tissue could not be determined using the global IL-15Rα KO mouse. Our data in the muscle-specific \textit{mIl15rafl/fl/Cre} mouse are consistent with a muscle-specific effect of IL-15Rα deficiency in altering the contractile properties and fatigue characteristics of skeletal muscle, although there were notable differences in the overall phenotype between this tissue-specific knockout mouse and the global IL-15Rα KO mouse. This muscle-specific effect of IL-15Rα deficiency is strengthened by the observation that the significant changes in fatigue resistance and contractile properties occurred despite no effects on cage activity and lower circulating IL-15 concentrations.

The \textit{Il15rafl/fl} mouse was created with loxP sites adjacent to exons 2 and 3 of the \textit{IL15RA} gene and has been used to knock these exons out selectively in immune cell populations as previously described (26). Exon 2 codes for the sushi-domain, which has a direct role in binding to IL-15, and exon...
3 codes for the linker region, which also contributes to high-affinity binding of IL-15 to IL-15Rα (5, 11). Based on our PCR analyses, MCK-Cre-mediated deletion of exons 2 and 3 occurred selectively within skeletal and cardiac muscle and not in the other tissues tested (i.e., kidney, liver, lung, spleen, and brain). Based on the known roles of exons 2 and 3 of the IL15RA gene, this would result in the inability of IL-15Rα to interact with IL-15 in muscle tissue only. Since it is appreciated that the role for IL-15Rα is high-affinity binding of IL-15 and not signal transduction, this results in a functional IL-15Rα knockout in muscle (2). Basal IL-15 and IL-15Rα levels in other cells and tissues, such as the immune system, likely remain unchanged in our animal model. In addition, the pattern of exon expression within the IL15RA gene was similar when comparing the mIl15rafl/fl/CreKO mouse and the global IL-15Rα KO mouse. Therefore, with respect to IL15RA gene expression, the molecular composition of the skeletal muscles in the muscle-specific mIl15rafl/fl/CreKO mouse is similar to that which has been described in the global IL-15RαKO mouse. However, we did observe that Il15 mRNA levels in the muscles of mIl15rafl/fl/CreKO mice were not different from controls, in contrast to our observations in the global IL-15RαKO mouse (31). These data suggest that selective deficiency of IL-15Rα in skeletal muscle is insufficient to drive greater transcription of Il15 mRNA and that the greater Il15 mRNA in muscles from the global IL-15Rα mouse occurs in response to a stimulus outside the muscle.

Despite the similar molecular composition of the IL15RA gene at the level of the skeletal muscle of these two transgenic mouse models, we observed significantly lower circulating IL-15 concentrations in the mIl15rafl/fl/CreKO mouse, in contrast to the elevated circulating IL-15 levels reported in the global IL-15RαKO mouse (16). Inasmuch as IL-15Rα has been suggested to be involved in secretion of IL-15 (3, 4), these observations suggest a considerable amount of IL-15Rα-dependent IL-15 secretion may be from skeletal muscle tissue, but IL-15Rα-independent IL-15 secretion mechanisms also exist. Our laboratory has previously proposed the hypothesis that IL-15 and IL-15Rα are regulators of the oxidative and fatigue properties of skeletal muscle in vivo, and that these alterations were driven by greater IL-15 concentrations in the circulation of global IL-15RαKO mice (31, 35), a hypothesis supported by data in muscle-specific IL-15 overexpressing mice (37). In the present study, although we observed changes in isolated skeletal muscle contractile and fatigue properties consistent with our previous data, the extent of these alterations were modest, and occurred despite lower circulating levels of IL-15. Superficially, these data do not support part of our proposed hypothesis. However, our blood collections for measuring circulating IL-15 occurred at the time of euthanasia.

**Fig. 4.** Fatigue curves in soleus muscles from mIl15rafl/fl/Cre+ mice and mIl15rafl/fl/Cre+ control mice. A: there was no significant effect of genotype on the fatigue curves obtained from the absolute forces produced by soleus muscles from female mIl15rafl/fl/Cre+ mice and female mIl15rafl/fl/Cre+ control mice. B: there was no significant effect of genotype on the fatigue curves obtained by calculating the fatigue index in soleus muscles from female mIl15rafl/fl/Cre+ mice and female mIl15rafl/fl/Cre+ control mice. C: there was a significant effect of genotype on the fatigue curves obtained from the absolute forces produced by soleus muscles from male mIl15rafl/fl/Cre+ mice and male mIl15rafl/fl/Cre+ control mice, such that lower absolute forces were produced by the mIl15rafl/fl/Cre+ mice. D: there was no significant effect of genotype on the fatigue curves obtained by calculating the fatigue index in soleus muscles from male mIl15rafl/fl/Cre+ mice and male mIl15rafl/fl/Cre+ control mice.
IL-15 from the muscles of the male mice. *Significant effect of genotype. #Significant effect of sex.

From an exercise performance perspective, there were differences in the overall exercise phenotype when we compared our data in the muscle-specific mIl15raflf/Cre mouse and the previously published data in the global IL-15RαKO mouse (16, 31). Male global IL-15RαKO mice ran over sixfold greater distances in a running wheel during the dark cycle compared with the B6129 background control mouse (31). These data were supported by others, who observed greater cage activity in female global IL-15RαKO mice using a photobeam system (16, 31). In contrast, there were no significant differences in running distance when comparing the mIl15raflf/Cre mouse to the mIl15raflf/Cre littermate control mice. The reasons for this difference may be due to the effects of knocking out IL-15Rα within the central nervous system, and altering IL-15 signaling in these tissues. IL-15Rα is normally expressed within the central nervous system, and IL-15 can cross the blood-brain barrier; moreover, manipulation of these molecules can affect circadian rhythms (14, 19, 51). Additionally, studies using the global IL15RαKO mouse suggest IL-15Rα can modulate activity and thermoregulation (16, 31) as well as normal anxiety behavior and antidepressive pathways (49, 50). Based on these data, it seems likely that knockout of IL-15Rα within the central nervous system can alter spontaneous cage activity. Therefore we speculate that the muscle phenotype observed in the global IL-15RαKO mouse was influenced by the greater amount of activity observed in this mouse (16, 31). Given our modest but significant changes in fatigue resistance in muscles from mIl15raflf/Cre mice in the absence of greater cage activity, we propose that muscle-specific knockout of IL-15Rα leaves a muscle in a trainable state.

Table 3. Contractile properties of soleus and EDL muscles from female and male Il15raflf/Cre+ and Il15raflf/Cre− mice

<table>
<thead>
<tr>
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<th>Female</th>
<th></th>
<th>Male</th>
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<th>Genotype</th>
<th>Sex</th>
<th>P Value</th>
<th>Interaction</th>
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<tr>
<td></td>
<td>mIl15raflf/Cre−</td>
<td>mIl15raflf/Cre+</td>
<td>mIl15raflf/Cre−</td>
<td>mIl15raflf/Cre+</td>
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<tr>
<td>Twitch force, mN</td>
<td>16.0 ± 5.35</td>
<td>14.7 ± 6.32</td>
<td>23.5 ± 5.21</td>
<td>15.5 ± 7.02 *</td>
<td>0.0131</td>
<td>0.0257</td>
<td>0.0721</td>
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<tr>
<td>TPT, ms</td>
<td>55.4 ± 6.60</td>
<td>58.0 ± 6.32</td>
<td>49.0 ± 7.38</td>
<td>46.9 ± 7.51</td>
<td>0.8975</td>
<td>0.0001</td>
<td>0.2653</td>
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<tr>
<td>½ RT, ms</td>
<td>76.2 ± 16.1</td>
<td>93.0 ± 31.3</td>
<td>67.0 ± 10.6</td>
<td>86.9 ± 39.9</td>
<td>0.0305</td>
<td>0.3589</td>
<td>0.8523</td>
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<tr>
<td>L0, mm</td>
<td>9.85 ± 0.44</td>
<td>10.3 ± 1.11</td>
<td>10.7 ± 0.48</td>
<td>10.4 ± 0.42</td>
<td>0.6420</td>
<td>0.0289</td>
<td>0.0593</td>
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<tr>
<td>Tetanus force, mN</td>
<td>109.6 ± 23.8</td>
<td>115.0 ± 42.3</td>
<td>152.9 ± 34.6</td>
<td>123.9 ± 41.4</td>
<td>0.2738</td>
<td>0.0190</td>
<td>0.1151</td>
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<td>Twitch/tetanus ratio</td>
<td>0.15 ± 0.04</td>
<td>0.12 ± 0.02</td>
<td>0.16 ± 0.05</td>
<td>0.12 ± 0.02 *</td>
<td>0.0071</td>
<td>0.6959</td>
<td>0.5184</td>
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<td></td>
<td></td>
<td>mIl15raflf/Cre+</td>
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<td>mIl15raflf/Cre−</td>
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<tr>
<td>Twitch force, mN</td>
<td>43.1 ± 11.6</td>
<td>42.8 ± 5.31</td>
<td>55.3 ± 10.5</td>
<td>45.0 ± 5.75*</td>
<td>0.0861</td>
<td>0.0207</td>
<td>0.1032</td>
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<tr>
<td>TPT, ms</td>
<td>28.5 ± 3.76</td>
<td>27.5 ± 7.07</td>
<td>27.5 ± 4.63</td>
<td>25.8 ± 5.15</td>
<td>0.4259</td>
<td>0.4259</td>
<td>0.8302</td>
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<td>½ RT, ms</td>
<td>46.9 ± 4.80</td>
<td>47.5 ± 7.07</td>
<td>38.8 ± 3.53</td>
<td>40.8 ± 5.15</td>
<td>0.4308</td>
<td>&lt;0.0001</td>
<td>0.6546</td>
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<td>L0, mm</td>
<td>10.9 ± 0.55</td>
<td>10.3 ± 0.72</td>
<td>11.9 ± 0.57</td>
<td>11.8 ± 0.55</td>
<td>0.0790</td>
<td>&lt;0.0001</td>
<td>0.2407</td>
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<tr>
<td>Tetanus force, mN</td>
<td>213.9 ± 39.00</td>
<td>221.2 ± 26.50</td>
<td>238.9 ± 53.28</td>
<td>224.3 ± 34.02</td>
<td>0.7741</td>
<td>0.2652</td>
<td>0.3845</td>
<td></td>
</tr>
<tr>
<td>Twitch/tetanus ratio</td>
<td>0.20 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.24 ± 0.03</td>
<td>0.20 ± 0.02*</td>
<td>0.0182</td>
<td>0.0143</td>
<td>0.1231</td>
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Values are means ± SD. TPT, time to peak tension; ½ RT, half-relaxation time; L0, optimal muscle length. *p < 0.05, Bonferroni post hoc mIl15raflf/Cre− mouse significantly different from mIl15raflf/Cre− mouse within same sex.
state, such that an increase in activity and/or training would exacerbate this phenotype and more closely resemble the phenotype first described in the global IL-15Rα/H9251 KO mouse (16, 31). Studies are currently being conducted to test this hypothesis.

From a muscle function and fatigue perspective, there was a difference in the degree to which fast muscles were resistant to contraction-induced fatigue in the two IL-15Rα/H9251 KO transgenic mouse models. In female muscle-specific mI15rafl/fl/Cre/H11001 mice, there was a significant, albeit modest, rightward shift of the fatigue curve compared with the curve generated in EDL muscles from female mI15rafl/fl/Cre/H11002 mice. This is in contrast to the larger shift observed in EDL muscles from the global IL-15Rα/H9251 KO mouse compared with the B6129 control mouse (31). However, skeletal muscles from both the global IL-15RαKO mouse and the mI15rafl/fl/Cre+ mouse displayed changes in mitochondrial density, mitochondrial DNA content, and molecular markers consistent with an increase in mitochondrial biogenesis (31, 33). Real-time qPCR experiments revealed that the cycle threshold for the mitochondrial genome-encoded COXII gene was achieved at 15 cycles in the mI15rafl/fl/Cre/H11001 mice and 16 cycles in the mI15rafl/fl/Cre/H11002 mice. A difference in 1 CT is equivalent to a twofold difference in gene expression, suggesting a twofold greater amount of mitochondrial DNA in the muscles from mI15rafl/fl/Cre/H11001 mice. These data are consistent with the hypothesis that manipulation of IL-15Rα/H9251 and IL-15 can influence mitochondrial changes, and support studies in other cell types showing a similar effect of manipulation of IL-15 and IL-15Rα on mitochondrial changes. The presence of IL-15 promoted mitochondrial biogenesis in CD8+ T cells as well as stimulated an increased expression of carnitine palmitoyl transferase (CPT1),

Fig. 6. Ex vivo isometric contractile properties of muscles from mI15rafl/fl/Cre+ mice and mI15rafl/fl/Cre- mice. A: representative twitch trace from soleus muscles. B: representative twitch trace from EDL muscles. C: there was a significant effect of genotype and gender on the absolute forces produced during an isometric twitch contraction of the soleus muscle. D: there was a significant effect of genotype as well as a significant statistical interaction on the specific force produced during an isometric twitch contraction of the soleus muscle. E: there was a significant effect of genotype on the twitch:tetanus ratio calculated from absolute forces produced by the soleus muscle. F: there was a significant effect of genotype on the 1/2 relaxation time of an isometric twitch contraction produced by the soleus muscle. G: there was a significant effect of genotype on the twitch:tetanus ratio calculated from absolute forces produced by the EDL muscle. *Significant effect of genotype. #Significant effect of sex.
which acts as the rate-limiting step in mitochondrial fatty acid oxidation (47). In addition, IL-15 has been shown to have a direct effect at stimulating lipolysis (1), and the global IL-15RaKO mice have a greater metabolic rate and higher resting body temperatures (16). These data demonstrate an association between states of increased IL-15, greater mitochondrial biogenesis, and increased lipid metabolism. Therefore, manipulation of IL-15 and IL-15Ra may represent an exercise mimetic strategy with the potential to stimulate exercise-like phenotypes in the muscles of those with limited exercise capacity via increases in circulating and/or tissue levels of IL-15 (37, 38).

Significant changes in isometric contractile properties were observed in the mIl15rafl/fl/Crenull mice, in a sex-specific manner, and were consistent with promoting a more oxidative muscle phenotype. The twitch:tetanus ratio, recognized as a marker of motor unit composition of a skeletal muscle (7), was significantly lower in EDL and soleus muscles from mIl15rafl/fl/Cre null mice compared with littermate mIl15rafl/fl/Cre null mice. Prior studies have demonstrated that lower twitch:tetanus ratio values in muscles are associated with the presence of slow motor units (9). These data are also supportive of the data in the global IL-15RaKO mouse (31) and the actinin 3 (ACTN3) KO mouse (10). Fast skeletal muscles from both the global IL15RaKO mouse and the ACTN3KO mouse behaved functionally more similar to an oxidative muscle, with increases in fatigue resistance and lower twitch:tetanus ratios (10, 31). In addition, the 1/2 RT during twitch contractions in soleus muscles was longer in mIl15rafl/fl/Cre null mice compared with littermate mIl15rafl/fl/Cre null mice. Longer twitch durations are characteristic of more oxidative muscles and reflect alterations at the level of calcium handling within the muscle fiber (28). The mechanisms leading to the sex-specific observations in muscle contractile properties are not exactly clear. However, prior studies have demonstrated sex-specific differences with manipulation of IL-15 (40). For example, muscle-specific overexpression of a secretable form of IL-15 inhibited fat deposition differentially in male and female mice, with fat deposition lower in the male mice. Additionally, female transgenic mice in this study showed greater deposition of lean body mass, compared with male mice (40). Interestingly, ingestion of megestrol acetate, a synthetic progestin, led to increased circulating IL-15 in elderly men, suggesting IL-15 levels may be regulated via sex steroids (20). Our laboratory has previously demonstrated that single nucleotide polymorphisms (SNPs) in the IL15 and IL15RA genes are associated with muscle phenotypes and markers of the metabolic syndrome in a sex-specific manner (32). Although a direct relationship between the observations in the present study and these previously published reports is not possible, collectively the data support the notion that IL-15 expression and/or activity is regulated differentially in males and females. Additional research is required to delineate the mechanisms leading to these sex-specific effects of IL-15 manipulation on exercise and muscle phenotypes.

Collectively, the data in the muscle-specific mIl15rafl/fl/Cre null mouse support a direct effect of functional deficiency of IL-15Ra in skeletal muscle in promoting changes toward a more oxidative muscle phenotype, likely due to alterations in mitochondrial density and/or function. However, targeting skeletal muscle for the tissue-specific deletion of IL-15Ra did not promote a greater amount of spontaneous cage activity or greater circulating IL-15. Therefore, the muscle-specific changes in fatigue and contractile properties observed in the mIl15rafl/fl/Crenull mouse model do not reflect all of the potential contributing mechanisms for the exercise and muscle function phenotype that we previously observed in the global tissue IL-15RaKO mouse (16, 31). The lack of an observed increase in circulating IL-15 in the mIl15rafl/fl/Cre null mouse likely contributed, in part, to this result. In addition, the effects of IL-15Ra modulation in the CNS likely contributed to the greater amount of cage activity in the global IL-15RaKO mouse. The effects of exercise to induce greater concentrations of circulating IL-15, and possibly work in conjunction with the muscle phenotype of the muscle-specific mIl15rafl/fl/Cre null mouse, as well as mechanisms for the sex-specific characteristics in these mice, remain to be explored.

ACKNOWLEDGMENTS

We acknowledge Drs. B. Teng and J. Mustafa for assistance with the real-time qPCR experiments performed in this manuscript.

GRANTS

This work was partially supported by the West Virginia University Research Development Grant (E. E. Pistilli).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

IL15RA Deficiency and Muscle Contractile Properties • O’Connell G et al.


