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From Anabolic to Oxidative: Reconsidering the Roles of IL-15 and IL-15Rα in Skeletal Muscle

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Abstract

Interleukin-15 (IL-15) and its receptor, IL-15 receptor-alpha (IL-15Rα), are suggested to function in determination of skeletal muscle phenotypes, with IL-15 originally proposed as an anabolic cytokine. This review will focus on recent work demonstrating that manipulation of IL-15 and IL-15Rα *in vivo* promotes changes in exercise capacity, muscle fatigue, and gene expression indicative of a more oxidative skeletal muscle phenotype.

Keywords

Cytokines; myokines; fatigue; oxidative muscle; atrophy; hypertrophy

INTRODUCTION

The term “myokine” has been used in reference to cytokines that are expressed and secreted from skeletal muscle to interact in paracrine or endocrine fashions with other cell and tissue types (20). In contrast to factors such as IL-6, which are secreted in high concentrations from skeletal muscle (20), biologically relevant levels of IL-15 have, until recently, been difficult to measure in human and particularly in animal models (34). Furthermore, the actions of IL-15 in cell culture models may not accurately represent actions that occur *in vivo*, potentially due to the observation that IL-15 expression and translation are regulated by complex intracellular and extracellular interactions with the alpha subunit of the trimeric IL-15 receptor (IL-15Rα) (3). Although the exact mechanisms of action of IL-15 signaling within skeletal muscle tissue have not been characterized, this review will report known

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effects of IL-15 and IL-15Rα modulation on skeletal muscle phenotypes and point to critical areas in need of future investigation. To begin to determine the roles of these molecules in skeletal muscle structure and function *in vivo*, we have utilized mouse models to over-express IL-15 transgenically as well as to delete ("knock out") IL-15 and IL-15Rα (23, 28). Collectively, our data have led to the hypothesis that IL-15 and IL-15Rα are novel regulators of the oxidative and fatigue properties of skeletal muscles *in vivo*, which contrasts with our original observations that IL-15 is an anabolic factor that promotes contractile protein accumulation and regulates protein synthesis/degradation rates, which was observed in muscle cell culture experiments (31). The experimental basis for this hypothesis, its implications for further research and clinical utility, and potential mechanisms of IL-15 action in skeletal muscle are presented in this review.

MOLECULAR REGULATION OF IL-15 AND IL-15Rα

IL-15 was discovered in 1994, and characterized as a T-lymphocyte growth factor based on its ability to mimic the actions of interleukin-2 (IL-2) with respect to cytotoxic T-lymphocyte proliferation and survival (10). In addition to shared actions on T-lymphocytes, IL-2 and IL-15 both utilize identical beta and gamma chain (IL-2Rβ, γc) components of their cell-bound trimeric receptors along with unique IL-2Rα and IL-15Rα subunits (9). IL-2Rα and IL-15Rα exhibit sequence homology, but exhibit binding specificity for their respective ligands (9). IL-2 and IL-15 are also structurally similar, containing four antiparallel alpha-helices, but do not exhibit sequence homology (10). Because of the structural similarity, both cytokines can signal through IL-2Rβ/γc heterodimeric complexes, or through heterotrimeric receptors which contain the respective specific alpha subunits and therefore are specific to each cytokine (9). These two cytokines differ considerably in cell type and tissue expression; while IL-2 expression is restricted to activated T-cells, IL-15 has a wide cell and tissue distribution including particularly high mRNA expression within skeletal muscle (10). Similarly, IL-15Rα has a wide tissue distribution (9), suggesting that the roles of IL-15 may extend beyond the immune system.

Despite widespread constitutive expression, IL-15 mRNA often does not correlate with protein expression (34). Initial analysis of the IL-15 gene determined that IL-15 expression was regulated at multiple levels based on the composition of the 5′ UTR, the unusually long signal peptide, and the C-terminus of the mature protein (1). IL-15 is inefficiently secreted due to its unusual signal sequence; however, IL-15 is biologically active and present in human serum (2). Recently, it has been demonstrated that a complex composed of IL-15 and IL-15Rα is formed within cells, and because IL-15Rα possesses an efficient signal sequence, this complex can then either be secreted or anchored in the cell membrane. The latter process has been termed trans-presentation, and provides a mechanism whereby IL-15 bound to IL-15Rα in the cell membrane can be presented to neighboring cells that express the IL-2Rβ and the γc (5). When IL-15 is complexed to the soluble isoform of IL-15Rα, this complex serves to stabilize IL-15 and modulate IL-15 bioavailability (3). The inability of some antibody preparations to detect IL-15/soluble IL-15Rα complexes provides an explanation for the reported difficulties in detecting IL-15 protein in biological samples (34). Moreover, the diversity in membrane bound and soluble isoforms of IL-15Rα, as well as complexed and uncomplexed IL-15 and IL-15Rα species, introduces further complexity in

the signaling possibilities of IL-15 and IL-15Rα. The signaling pathways activated by IL-15 binding, determined from cell culture studies, include the activation of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway (13); however, the specific IL-15 signaling pathways activated in skeletal muscle under different physiologic conditions remain to be elucidated.

IL-15 ACTIONS IN MYOGENIC CELL CULTURE

The seminal study of IL-15 by Grabstein et al. (10) demonstrated that IL-15 mRNA was highly expressed in human muscle tissue. Based on this observation, a potential role for IL-15 in skeletal muscle tissue was explored by determining the effects of exposure to recombinant IL-15 on immortalized and primary skeletal myogenic cultures (7, 27, 31). Several studies using murine, human, and bovine skeletal myogenic cultures indicated that addition of recombinant IL-15 induced a hypertrophied phenotype in myotubes (immature muscle fibers) and increased contractile protein accumulation (7, 27, 31). Importantly, in these studies IL-15 did not stimulate proliferation or differentiation of muscle precursor cells (myoblasts), but rather acted as a hypertrophic factor in cultured myotubes themselves. This mode of action differs from other muscle anabolic factors such as insulin-like growth factor, whose actions are largely due to stimulation of myoblast activities (7, 27, 31). However, one study showed IL-15 could induce myoblast differentiation in conditions mimicking aging, in which insulin-like growth factor signaling was reduced (30), suggesting a complex relationship between these two factors. In a follow-up study, we up-regulated IL-15 expression in the immortalized mouse C2C12 cell line using a replication-deficient retroviral vector (27); this study indicated IL-15 increased protein synthetic rates and decreased protein degradation rates in myotubes in the absence of effects on myoblasts. These findings led us to propose that IL-15 was an anabolic factor for skeletal muscle, with potential therapeutic use for muscle wasting conditions (27, 30, 31). These findings also suggested that muscle cells could actively secrete and respond to IL-15 in an autocrine/paracrine fashion. These studies provided the rationale to treat animal models with IL-15, with the goal to attenuate muscle wasting in various atrophic and/or pathologic conditions (27). In particular, the ability of IL-15 to inhibit myotube protein degradation suggested it may be of utility in treating the muscle wasting characteristic of cancer cachexia, muscular dystrophies, or aging (i.e. sarcopenia).

IL-15 AND IL-15Rα ACTIONS *IN VIVO*

IL-15 Expression in Muscle Tissue

Analysis of multiple human tissues indicated a comparatively greater IL-15 mRNA abundance within muscle tissue (10). The observation of preferential expression of IL-15 mRNA and protein within muscle tissue was supported in studies of biopsy samples from athletes (18). This led us to determine whether IL-15 mRNA expression was regulated by conditions that either promote muscle hypertrophy or muscle atrophy in experimental animal models. We examined IL-15 mRNA expression in response to the muscle atrophy induced by acute hindlimb unloading in young adult and aged rats. Our data demonstrated that IL-15 mRNA was greater in muscles following unloading in young and in aged muscles, although

there were differences between muscles with differing fiber type composition (24). Using a quail model of wing weighting and subsequent unloading, we demonstrated that IL-15 mRNA expression was not affected by the hypertrophic stimulus of wing weighting, but was greater in young adult and aged quail muscles following the atrophic stimulus of wing unloading. This study also demonstrated that skeletal muscle expressed detectable levels of all three components of the IL-15R, including IL-15Rα, IL-2Rβ, and γc (24). Based on our previous data in myogenic cultures, we speculated that IL-15 mRNA accumulation was a molecular attempt of skeletal muscle to counteract pro-atrophic conditions, although the restrictions on IL-15 translation limited the ability of muscle-derived IL-15 to spare muscle mass.

Marzetti et al. (14) examined IL-15 and IL-15Rα gene expression and protein in the mixed fiber gastrocnemius muscles in young adult and aged rats in response to caloric restriction. IL-15 mRNA expression was unchanged in the gastrocnemius muscles of aged rats. However, IL-15 protein was lower in aged muscle, along with IL-15Rα gene expression. Interestingly, caloric restriction partially attenuated these aging-related reductions in IL-15 and IL-15Rα. Similar findings were reported by us in aging mouse muscles (29). Collectively, these data demonstrated atrophic conditions induce an altered expression pattern of IL-15 and IL-15Rα within skeletal muscle. However, differential responses of IL-15 mRNA were noted in response to sarcopenia versus disuse atrophy; moreover, as noted above, discordance between IL-15 mRNA and protein expression was noted in aging muscle.

Experimental Administration of IL-15

A number of studies by us and others were conducted in which IL-15 was provided therapeutically to experimental animals in an attempt to modulate muscle mass under normal and atrophic conditions (4, 6, 11, 22). Two studies performed in cachetic rats implanted with tumor cells demonstrated that seven days of recombinant (r) IL-15 administration spared muscle mass, and was associated with a blunting of the apoptotic pathway downstream of tumor necrosis factor-alpha (TNF-α) (4, 6). Moreover, in accord with studies in myogenic cell cultures, rIL-15 inhibited muscle protein degradation in both cachetic and healthy (non-tumor-bearing) rats. These studies appeared to provide an extension of our initial studies in myogenic cultures indicating that IL-15 had anabolic properties (27, 30, 31). However, a closer examination of these studies does not support this speculation. Although IL-15 administration into cachetic animals led to a sparing of muscle mass, this was not the case for the healthy (non tumor-bearing) animals. Healthy animals in these studies that received rIL-15 exhibited no changes in muscle mass, possibly because protein synthesis in the healthy animals (but not in the cachetic animals) was decreased by IL-15, balancing the decrease in protein degradation. Specifically, in healthy rats, overall body mass was not significantly different between the control rats and the IL-15 injected rats at the time of euthanasia (4). In addition there were no significant differences in the wet weights of the gastrocnemius, soleus, or tibialis anterior skeletal muscles from control and IL-15 injected rats. One could speculate that this may have been due to an IL-15 treatment duration of only 7 days. However, similar results have been observed with longer exposures of rIL-15 into control animals and in transgenic mice with constitutive over-expression of

IL-15. Moreover, short-term treatment of IL-15 functioned to reduce adipose tissue mass, providing a positive control for IL-15 bioactivity and bioavailability (28), and consistent with cell culture studies which showed IL-15 inhibits adipocyte differentiation *in vitro* (25).

We studied the potential of rIL-15 to attenuate pro-apoptotic signaling that occurs in skeletal muscles of aged rats (22). The experimental design of this study included both young adult and aged rats implanted with mini-osmotic pumps delivering rIL-15 or saline vehicle for 14 days. Notably, IL-15 treatment had no effect on overall body weight or muscle weights in young or old rats (22). Harcourt et al. (11) utilized mini-osmotic pumps to increase rIL-15 levels in control and dystrophic (*mdx*) mice for 4 weeks. This study also showed no differences in body weight following rIL-15 administration in control or *mdx* mice. However, the authors did report a significant increase in the specific force of diaphragm strips from *mdx* mice treated with rIL-15, and an increase in fiber cross-sectional area of toxin-treated muscles. These studies demonstrated that IL-15 acted to spare muscle in wasting conditions, but that IL-15 was not sufficient to induce anabolic effects in healthy control animals. It is possible that healthy animals exhibit counter-regulatory mechanisms to IL-15 action which are not present in myogenic cell cultures.

Collectively, the results of multiple studies in which wild type control animals were exposed to exogenous IL-15 demonstrate minimal effects on either overall body mass or the mass of individual skeletal muscles. To date, the muscle-sparing and/or anabolic effects of IL-15 *in vivo* have only been observed in cachetic rats and in a mouse model of neuromuscular disease (4, 6, 11). Therefore, a distinction should be made between the muscle-sparing effects of IL-15 observed in catabolic conditions (4, 6), and the anabolic effects of IL-15 noted in myogenic cultures but not in healthy rodents (27, 30, 31). If the actions of IL-15 were anabolic, this effect should be observed in body and skeletal muscle masses in these various models. Given these observations, we propose that the myokine IL-15 should not be considered an anabolic cytokine with regards to actions *in vivo*, despite our initial observations in myogenic cultures.

Genetic Manipulation of IL-15 *In Vivo*

In order to study the effects of IL-15 in a longer-term model, we constructed two lines of transgenic mice that over-expressed IL-15 from a strong muscle-specific promoter, a modified human skeletal actin (HSA) promoter with activity restricted to skeletal muscle tissue (28). The IL-15 transgene used to construct one line of IL-15 transgenic mice retained the inefficient native IL-15 long signal peptide (LSP) sequence, hence the resulting transgenic mouse line was termed HSA-natLSP-IL15. Although HSA-natLSP-IL15 mice expressed high levels of intramuscular IL-15 mRNA and protein, circulating IL-15 concentrations did not differ from controls. HSA-natLSP-IL15 mice exhibited no detectable phenotypic differences from littermate controls. In another transgenic mouse line, HSA-IL2SP-IL15, the inefficient native IL-15 LSP was replaced with a more efficient signal peptide sequence from IL-2; consequently, HSA-IL2SP-IL15 mice exhibited both high intramuscular and circulating IL-15 levels. Compared to littermate controls, HSA-IL2SP-IL15 mice exhibited reduced visceral and subcutaneous fat deposition, were more insulin-sensitive, and were resistant to diet-induced obesity (26, 28). No difference in circulating

levels of IL-2, IL-6, or tumor necrosis factor-alpha (TNF-α) were observed between controls and HSA-IL2SP-IL15 mice, but reflective of lower adiposity, circulating leptin levels were significantly lower in these transgenic mice (26, 28). Comparison of the two lines of IL-15 over-expressing mice which differed in circulating IL-15 levels demonstrated that IL-15 secretion into the circulation was necessary for the effects of IL-15 on fat deposition *in vivo*. These findings confirmed the identity of IL-15 as an authentic myokine, and were consistent with previous *in vitro* and short-term *in vivo* studies showing a direct effect of IL-15 on adipose tissue (25).

However, while the HSA-IL2SP-IL15 transgenic mouse line had a higher percentage of lean tissue per gram body weight, this was solely due to a reduction in body fat, as no increase in absolute lean tissue mass was detected. Minimal effects on muscle mass were observed, limited to a slight increase in the weight of the predominately slow/oxidative soleus muscle and a slight decrease in the weight of the predominately fast/glycolytic extensor digitorum longus (EDL) muscle. Indeed, while differences in physiological properties of these muscles compared to controls were not observed (23), a decrease in expression of the fast mRNA isoform of troponin I and an increase in expression of the slow mRNA isoform of troponin I was observed in both fast and slow muscles in HSA-IL2SP-IL15 mice compared to controls. Moreover, other markers of oxidative metabolism, including SIRT1 and UCP2 mRNA, were up-regulated in muscles in this transgenic line (26). These findings indicate clearly that, although IL-15 was secreted and biologically active in this line (as evidenced by its effects on adipose tissue deposition), muscle hypertrophy was not induced. However, inasmuch as slow/oxidative muscle fibers are smaller than fast/glycolytic fibers, a putative anabolic effect of IL-15 *in vivo* may have been confounded by a transition to more oxidative muscle fibers in this model. Additionally, as slow/oxidative muscle fibers are more resistant to atrophy than fast/glycolytic fibers, the shift toward a more oxidative muscle phenotype may explain *in vivo* findings summarized above that IL-15 administration can inhibit muscle wasting in atrophic conditions, but is not anabolic in healthy animals.

Because of the discordance between the hypertrophic effects of IL-15 in muscle cell cultures and studies concerning IL-15 action *in vivo,* little progress has been made in establishing a clear mechanism for IL-15 action in muscle. We believe this situation is due to the confounding effect of IL-15 on muscle phenotype *in vivo*. A reliance on preservation of muscle weight alone as an assay for inhibition of sarcopenia or muscle wasting, without a concomitant analysis of muscle phenotype, may be responsible for the failure to pursue IL-15 as a potential therapeutic avenue.

Genetic Manipulation of IL-15Rα *In Vivo*

In lymphoid systems, IL-15Rα regulates secretion, stability and bioavailability of IL-15 (3). Therefore, genetic manipulation of IL-15Rα *in vivo* was also examined using a total tissue IL-15Rα knockout mouse (B6;129X1-*Il15ra*^{tm1Ama}/J). We analyzed exercise and muscle phenotypes of these mice at 2–3 months of age using wheel running as a surrogate measure of exercise capacity as well as *ex vivo* muscle contractile techniques, and compared these parameters to B6129SF2/J background control mice (23). Mice were provided access to cage-mounted running wheels during the 12 hour dark cycle for 3 consecutive nights. The

difference in wheel running distance covered during the data collection period was striking, with IL-15RαKO mice covering a 6-fold greater distance when compared to B6129 control mice (Figure 1a). These data were validated through the observation of a greater number of ambulatory counts using an open field photobeam system in a separate cohort of IL-15RαKO mice, and supported data from a separate laboratory (12). Cage activity and running distances were also greater than those of mice that lacked IL-15 (i.e. IL-15KO mice) and their corresponding BL/6NTac background control mice (Pistilli and Khurana, unpublished observations). These data suggested that knockout of IL-15Rα resulted in a mouse that had an increased capacity for cage activity and/or exercise, and that this phenotype was specific to IL-15RαKO mice, as opposed to IL-15KO mice.

The dramatic difference in wheel running performance in IL-15RαKO mice led us to analyze the *ex vivo* isometric contractile properties and fatigue characteristics of muscles from these mouse strains. Muscle fatigue was examined using a repeated stimulation protocol, with the fatigue index plotted as the percent difference in force during repeated contractions. A significant rightward shift was observed in the fatigue index curve of fast EDL muscles from IL-15RαKO mice compared to controls, demonstrating maintenance of force production over time (i.e. an increase in fatigue-resistance; Figure 1b). This response was not observed in HSA-IL2SP-IL-15 mice or IL-15KO mice in age-matched cohorts. Isometric twitch and tetanus forces of fast EDL muscles from IL-15RαKO mice were significantly lower than controls. Interestingly, the twitch/tetanus ratio which serves an indirect marker of the motor unit composition of a muscle, was significantly lower in the fast EDL muscles from IL-15RαKO mice, consistent with a transition to a more oxidative muscle phenotype. There were no differences in any of these measures in the slow soleus muscles from these mouse strains, providing more evidence for an oxidative transition with knockout of IL-15Rα.

These experiments were followed by histological and molecular analyses, which supported the exercise and *ex vivo* contractile data. Fast EDL muscles from IL-15RαKO mice had a greater number of smaller sized muscle fibers and a greater nuclear density compared to EDL muscles from control mice. Histological examination of succinate dehydrogenase (SDH) and gene levels of citrate synthase were greater in muscles from IL-15RαKO mice, suggestive of increased mitochondrial density and/or activity. Gene and protein levels of PPARδ and PGC-1α were also greater in muscles from IL-15RαKO mice, providing more evidence for mitochondrial alterations in IL-15Rα deficient muscles.

Interestingly, muscles from IL-15RαKO mice expressed greater levels of IL-15 mRNA, and systemic IL-15 levels have been reported to be greater in these mice (23, 35). These observations suggest that, rather than interfering with IL-15 signaling (which can occur in the absence of IL-15Rα), deletion of IL-15Rα may release IL-15 into the circulation by eliminating surface-associated IL-15/IL-15Rα complexes and additionally derepress negative feedback of IL-15 mRNA expression. This hypothesis would explain the phenotypic similarities of HSA-IL2SP-IL15 mice and IL-15RαKO mice, which both exhibit elevated circulating IL-15 levels and display pro-oxidative muscle phenotypes. Subtle differences in muscle physiology and body composition between these two models are nevertheless observed; thus, experiments are ongoing to acquire more direct measures of

mitochondrial density and activity in muscles from these two models to distinguish this hypothesis from that of a direct effect of IL-15Rα deficiency in altered muscle phenotypes.

IL-15 AND EXERCISE

The extent that IL-15 expression is modulated by exercise remains undetermined. Studies have examined IL-15 mRNA and protein levels in skeletal muscle and serum in response to different types of exercise (aerobic vs. resistance training) and in both athletes and non-athletes (16–19, 33). These studies provide conflicting results on IL-15 levels, which are complicated by differing methods for IL-15 analysis, exercise protocol, and subject populations. With regard to aerobic exercise, Nieman et al. (18) measured IL-15 mRNA levels from vastus lateralis biopsy samples obtained immediately after a 3h treadmill run at 70% VO_{2max} in experienced marathon runners. Although IL-15 mRNA was the highest of the cytokine mRNAs measured at baseline, there were no changes in IL-15 mRNA concentrations immediately after the 3h run. Ostrowski et al. (19) measured plasma cytokines in ten athletes before, during, and after a 2.5h treadmill run at 75% VO_{2max} and reported no changes in plasma IL-15. In contrast, Tamura et al (33) reported a significant increase in serum IL-15 10 minutes after a 30-minute treadmill run at 70% VO_{2max} in untrained young men; serum IL-15 levels returned to baseline within 2h, suggesting the timing of muscle biopsy or serum sampling could miss a transient post-exercise rise in IL-15. The circulating concentration of IL-15 in the two latter studies ranged from 1.7 $pg.ml^{-1}$ to 3.9 $pg.ml^{-1}$, thus a putative post-exercise spike in IL-15 is modest compared to post-exercise increases in circulating concentrations of IL-6, which can be up to 100-fold (20).

Changes in IL-15 levels in response to resistance training are also conflicting. For example, Nieman et al. (17) measured IL-15 mRNA levels from vastus lateralis biopsy samples obtained immediately after a 2h resistance training bout in strength-trained subjects. Similar to their prior study with endurance exercise, IL-15 mRNA levels were greatest among cytokine mRNAs, but were unchanged immediately after the exercise bout. However, Riechman et al. (32) measured IL-15 protein in serum samples following an acute bout of resistance exercise before and following 10 weeks of resistance training in untrained subjects. Acute resistance exercise caused a significant increase in circulating IL-15, although this increase was not affected by the 10 weeks of resistance training. Nielsen et al. (16) measured IL-15 mRNA and protein in muscle biopsy samples from physically active males. A preferential expression of IL-15 mRNA was found in muscles comprised of a greater percentage of type II muscle fibers, although no differences were noted in protein levels among muscles. This study also analyzed IL-15 mRNA and protein from vastus lateralis biopsy samples as well as circulating IL-15 levels following an acute bout of resistance training. IL-15 mRNA was 2-fold greater at 24h after resistance exercise, although IL-15 protein in muscle and the circulation was not changed. Collectively, these data do not provide a conclusive effect of exercise on IL-15, perhaps due to differences in mode of exercise and/or subject population. However, these results underscore the above-mentioned discordance between IL-15 mRNA expression and protein levels, suggesting a post-translational mechanism for IL-15 secretion, perhaps involving IL-15Rα.

POTENTIAL MECHANISMS OF ACTION FOR IL-15 IN MUSCLE

As noted above, the molecular pathways by which IL-15 acts on skeletal muscle tissue have not been characterized, as attention has been focused on the expression and effects of this cytokine on skeletal muscle *in vitro* and *in vivo*. Potential signaling pathways impacted by IL-15 include: 1) inhibition of catabolic TNF-α signaling; 2) direct induction of intracellular factors that control muscle protein synthesis and degradation; and 3) induction of intracellular mediators of oxidative metabolism such as the sirtuin, PPARδ, and PGC-1α pathways (Figure 2). In regard to the first possibility, IL-15 expression and TNF-α levels are negatively correlated in aging rodent muscles (14). Moreover, some studies in non-muscle cells have suggested the activated IL-15Rα and the TNF receptor can competitively inhibit one another (21). This mechanism would explain the IL-15-induced inhibition of muscle protein degradation and apoptosis. However, this idea is unlikely given that mice which lack IL-15Rα and have elevated levels of IL-15 exhibit phenotypic similarity to IL-15TG mice, as reviewed above. The second possible mechanism, a direct induction of intracellular modulators of protein dynamics, is attractive but would explain only the effects of IL-15 on protein dynamics. Moreover, unpublished studies from our laboratories have failed to detect any upregulation by IL-15 of mRNAs coding for myostatin, the E3 ubiquitin ligases MuRF1 and MAFbx (atrogen-1), or FOXOs 1–4 (Quinn, unpublished observations). As this examination was at the mRNA level only, further work is required to determine changes in protein expression before a stronger conclusion can be reached.

In contrast, some published evidence supports the third hypothesis, that IL-15 stimulates expression of pro-oxidative metabolic mediators such as SIRT1, PPARδ, and PGC-1α in skeletal muscle cells. In cultured myogenic cell lines, IL-15 induced metabolic changes were shown to be dependent on PPARδ (8). We showed that SIRT1 mRNA expression was elevated in soleus muscles from IL-15TG mice (26). Moreover, muscles from IL-15RδKO mice express greater levels of PPARδ and PGC-1α mRNAs (23). In transgenic mouse models, each of these factors have been shown to induce pro-oxidative shifts in skeletal muscle and in some cases confer protection from skeletal muscle atrophy (15). Inasmuch as expression of these factors are stimulated by exercise and other forms of physiologic stress (15), these findings are consistent with IL-15 as a component of the innate immune system (34) and the regulation of IL-15 with exercise (16–19, 32). Therefore, although there is some experimental support for this model, further work is needed to confirm this hypothesis.

CONCLUSIONS

Since the initial characterization of IL-15 as a T-lymphocyte growth factor with high mRNA expression within skeletal muscle (10), numerous experiments have been performed to determine a role for this cytokine in muscle tissue. These experiments have included incubating myoblasts and myotubes with differing levels of rIL-15 (7, 30, 31), increasing IL-15 expression in myogenic cultures through viral means (27), increasing the systemic levels of IL-15 through direct injections or osmotic pumps (4, 6, 11, 22), and altering IL-15 and IL-15Rα expression in genetic mouse models (23, 26, 28). The studies that have increased systemic levels of rIL-15 through intraperotineal injections or implantation of osmotic pumps showed no effects on overall body mass or muscle mass in healthy rodents,

although these delivery methods may not mimic how IL-15 appears in vivo (i.e. endocrine versus paracrine/autocrine). However, the data in our IL-15TG mice, which have high levels of muscle IL-15 as well as in the circulation, also showed no anabolic effects on muscle mass. The most recent data we have collected in genetic mouse models highlights a previously unrecognized role of IL-15 and IL-15Rα in defining and/or altering the oxidative phenotype of skeletal muscles (23, 26, 28). This proposed role contradicts our original hypothesis that IL-15 was an anabolic factor for skeletal muscle (31). In this review, we have presented physiological data (i.e. wheel running, muscle contractile stimulation), molecular data, and muscle morphology data to support our conclusion. Therefore, we would like to suggest a new hypothesis whereby IL-15 and IL-15Rα are regulators of the oxidative and fatigue properties of skeletal muscle *in vivo* (Figure 3).

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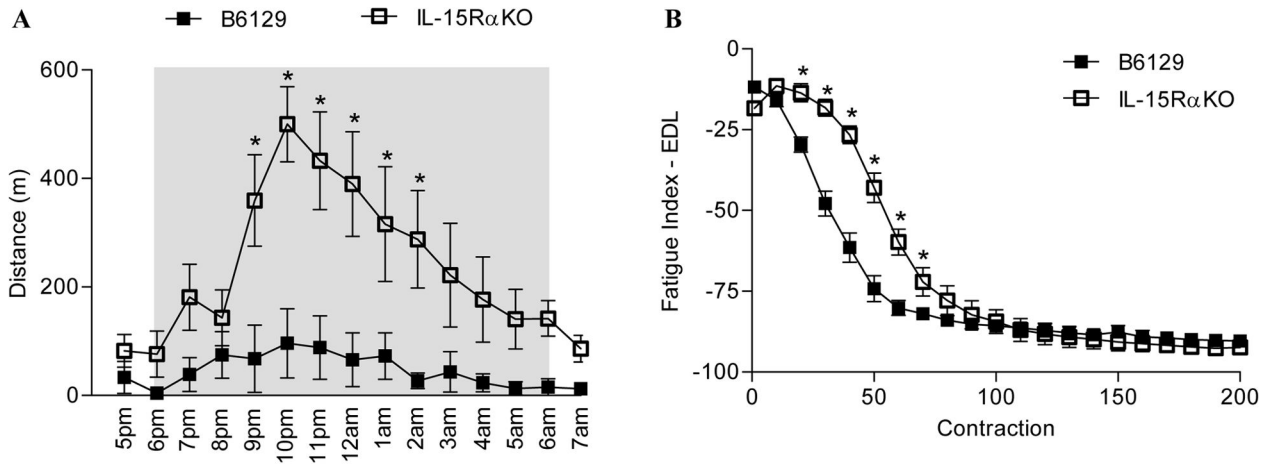

Figure 1. Wheel running and fatigue curves of muscles from IL-15RaKO mice

(A) IL-15RαKO mice and B6129 control mice were provided with a cage mounted running wheel to quantify overall activity levels. The total distance covered by IL-15RaKO mice was significantly greater than the control mice, and averaged a 6-fold difference during the total data collection period. **(B)** The fatigue index was quantified in fast EDL muscles from IL-15RaKO mice and B6129 control mice. The fatigue index curve from IL-15RaKO EDL muscles was shifted to the right compared to EDL muscles from B6129 control mice, indicating a maintenance of force production with repeated contractions (i.e. fatigue-resistance). *, $p<0.05$. (Reprinted from (23). Copyright © 2011 The American Society for Clinical Investigation. Used with permission.)

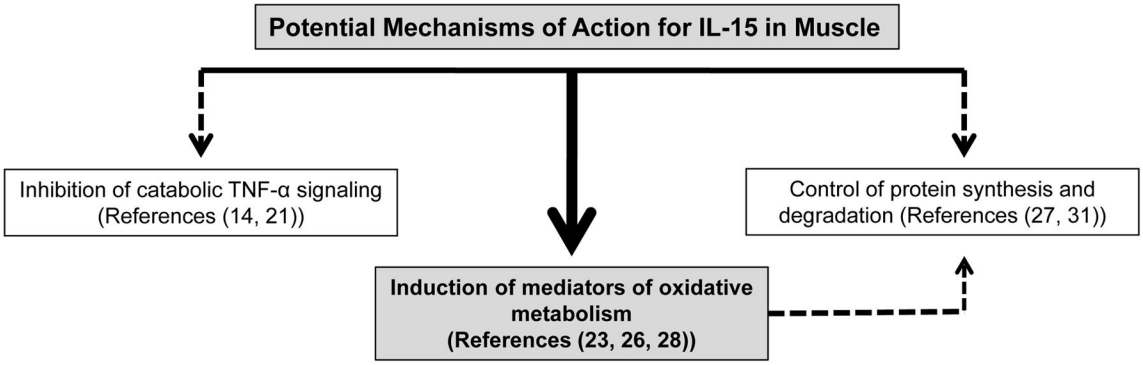

Figure 2. Potential mechanisms of action for IL-15 in muscle

Potential signaling pathways impacted by IL-15 include: 1) inhibition of catabolic TNF-α signaling; 2) induction of intracellular factors that control muscle protein synthesis and degradation; and 3) induction of intracellular mediators of oxidative metabolism such as the sirtuin, PPARδ, and PGC-1α pathways, with indirect effects on modulators of muscle protein synthesis and degradation. The most recent data collected by the authors suggest that the induction of oxidative mediators may be the most likely of these hypotheses. This hypothesis is supported by the strong evidence collected in 2 separate strains of mice, showing that there is up-regulation of SIRT1, PPARδ, and PGC-1α in the skeletal muscles of IL-15TG mice and IL-15RαKO mice. Additional studies are currently being pursued in our labs to validate this hypothesis.

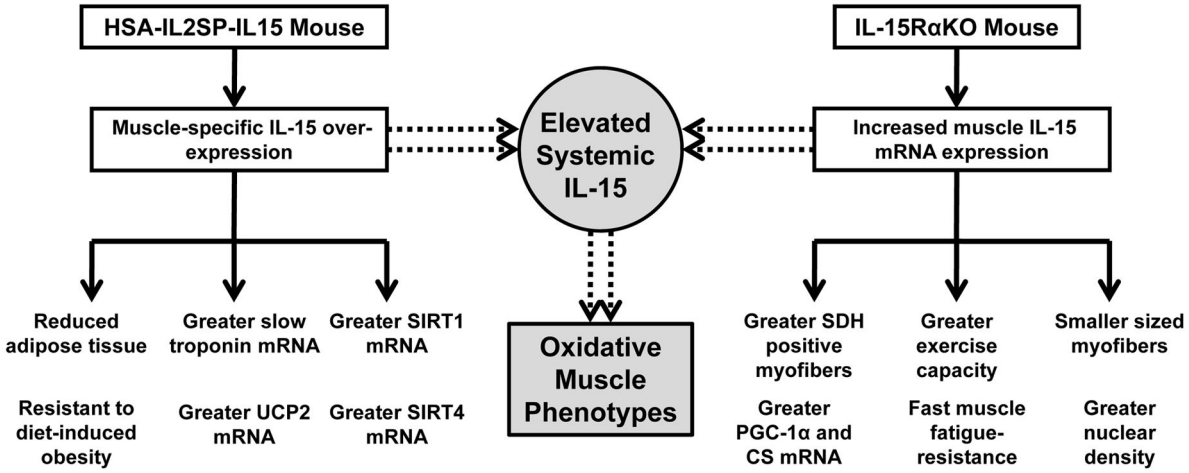

Figure 3. Proposed model for oxidative muscle phenotype with altered IL-15 and IL-15Rα in vivo

Circulating levels of IL-15 were greater in the muscle-specific HSA-IL2- IL15TG transgenic mouse and the IL-15RαKO mouse. The physiological changes in these different mouse strains, including longer treadmill running, greater running wheel activity, and greater fast muscle fatigue-resistance, suggested the skeletal muscles adopted a more oxidative phenotype. At the molecular level, these changes included greater expression of SIRT1 and slow troponin mRNA (HSA-IL2-IL15TG mouse) and greater expression of markers involved in mitochondrial biogenesis (IL-15RαKO mouse). Fast muscles also displayed a slow muscle morphology, including a shift toward smaller muscle fiber sizes and a greater nuclear density. Collectively, these changes occurred in the presence of serum IL-15 levels between 200pg.ml^{-1} and 600pg.ml^{-1}.