

**EFFECTS OF INTRINSIC AEROBIC CAPACITY, AGING AND  
PHYSICAL ACTIVITY ON INTERLEUKIN-15 PROTEIN LEVEL  
IN SERUM AND SKELETAL MUSCLE**

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## ABSTRACT

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Myokines are cytokines and other peptides produced in muscle that can transmit autocrine, paracrine or endocrine signals to other tissues. This is a potential way for exercise to carry out its beneficial effects on different parts of the body. Interleukin-15 (IL-15) is a myokine that has been shown to exert beneficial effects on multiple tissues: skeletal muscle, adipose tissue, bone and skin. Muscle-derived IL-15 signaling remains to be fully elucidated. It is known that the expression and signaling is affected by aging, exercise, and intrinsic aerobic capacity. The purpose of this study was to assess the effects of intrinsic aerobic capacity, aging and physical activity separately and in combination on muscle and serum interleukin-15 protein levels in rats. Two rat strains were used: high capacity runner (HCR) and low capacity runner (LCR) rats, which have been selectively bred based on intrinsic running capacity. They were divided into 3 subgroups (n=10 per group): adult rats (HCR/LCR), old rats (HCR-o/LCR-o) and old rats with running wheel (HCR-oR/LCR-oR), for a total of 6 groups. IL-15 protein levels were measured from serum with ELISA and from three different muscles with western blot: gastrocnemius, soleus and extensor digitorum longus (EDL). The selected antibody produced two different signals in the western blot, assumed to represent two different isoforms of IL-15, and both were quantified. Voluntary running distance in runner groups was measured with a computerized recording system. The results of the present thesis show that IL-15 protein level was increased in the HCR strain compared to the LCR strain in the gastrocnemius ( $P=0.014$ ) and EDL ( $P<0.001$ ) for separate isoforms. IL-15 was increased in the serum ( $P=0.010$ ), but reduced in the soleus ( $P=0.005$ ) for one isoform with aging, whereas running was found not to have any significant effects. Univariate analyses also showed no combinatory effects of strain\*age or strain\*running. The main finding of this study was that intramuscular IL-15 protein was increased in the HCR compared to the LCR strain, as hypothesized based on unpublished microarray data. However, HCR rats did not show elevated IL-15 in serum. These results suggest that IL-15 action could be responsible in part for explaining some of the differences between HCR and LCR rats, such as oxidative properties of different muscles or abdominal fat.

Key words: myokine, interleukin-15, intrinsic running capacity, aging, physical activity

## ABBREVIATIONS

AMPK	5' adenosine monophosphate-activated protein kinase
BSA	Bovine serum albumin
Cys	Cysteine
EDL	Extensor digitorum longus
ELISA	Enzyme-linked immunosorbent assay
HCR	High capacity runner
IGF-1	Insulin-like growth factor 1
IL-2	Interleukin-2
IL-2R $\beta$	Interleukin-2 receptor $\beta$
IL-2R $\gamma$	Interleukin-2 receptor $\gamma$
IL-15	Interleukin-15
IL-15LSP	Long signal peptide interleukin-15
IL-15R $\alpha$	IL-15 receptor $\alpha$
IL-15SSP	Short signal peptide interleukin-15
JAK	Janus kinase
LCR	Low capacity runner
MAPK	Mitogen-activated protein kinase
mRNA	Messenger ribonucleic acid
PI3-kinase	Phosphatidylinositol 3 kinase
PVDF	Polyvinylidene fluoride
PPAR $\delta$	Peroxisome proliferator-activated receptor $\delta$
SIRT 1	Sirtuin 1
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
STAT	Signal transducer and activator of transcription
TBS-T	Mixture of tris-buffered saline and tween 20
TNF- $\alpha$	Tumor necrosis factor $\alpha$
UCP2	Uncoupling protein 2

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# 1 INTRODUCTION

It is well known that exercise has beneficial effects on the human body. Regular exercise is able to cause improvements in insulin sensitivity, metabolic processes, blood pressure and reduce inflammation. This is, in part, mediated through changes in gene or protein expression in different types of cells. (Henningsen et al. 2010.) In recent years it has been shown that skeletal muscle has properties of an endocrine organ. Skeletal muscle is able to produce and secrete proteins that systemically influence biological processes in other tissues. These proteins have been named myokines. The secretion of myokines is assumed to be one way through which the beneficial effects of physical activity are carried out. Myokine signaling can be autocrine, paracrine, or endocrine. (Pedersen 2011.) In 2010, 635 different proteins were shown to be secreted by skeletal muscle, which could potentially be signaling mediators to other cells and tissues. 188 of these were significantly regulated during myogenesis (formation of muscle tissue). The secretion profile was termed skeletal muscle secretome. (Henningsen et al. 2010.) Roca-Rivada *et al.* (2012) compared secretome characteristics between slow-oxidative and fast-glycolytic muscles. They found 19 proteins that were differently secreted. 1 week of endurance exercise changed the secretion profile for both muscle types. This shows that the secretome of skeletal muscle has a dynamic nature that depends on factors such as exercise and muscle fiber type. Different signals can be given to other tissues in different situations.

This literature review will focus on interleukin-15 (IL-15), which is one of the myokines found in the skeletal muscle secretome (Henningsen et al. 2010). As a myokine, IL-15 has been shown to induce changes in muscle tissue itself (Pistilli & Quinn 2013), adipose tissue (Alvarez et al. 2002), bone (Quinn et al. 2009) and skin (Crane et al. 2015). IL-15 mRNA is elevated in the muscle of rats with high intrinsic aerobic capacity (Kivela et al. 2010, unpublished finding).

The primary purpose of this study was to assess the relationship of intrinsic aerobic capacity and IL-15 protein in different muscles and serum. A secondary purpose was to assess the

effects of aging and physical activity in this framework. IL-15 protein levels were measured in rat serum and the gastrocnemius, soleus and extensor digitorum longus (EDL) muscles.

## **2 INTERLEUKIN-15 BIOLOGY**

### **2.1 Overview**

Interleukin-15 was discovered in 1994 by two independent groups (Burton et al. 1994, Grabstein et al. 1994). Structurally IL-15 is part of the 4 alpha-helix bundle cytokine family. This family includes cytokines (such as IL-2, IL-3, IL-4, IL-6, IL-21) and other signal molecules such as growth factors and hormones (e.g. erythropoietin, human growth hormone, prolactin). (Budagian et al. 2006.)

IL-15 is expressed in many different tissues. This includes monocytes, dendritic cells, keratinocytes, epidermal skin cells, fibroblasts, different types of epithelial cells, nerve cells as well kidney, placenta, lung, heart, and also skeletal muscle tissue (Budagian et al. 2006). mRNA expression is particularly high in skeletal muscle, as shown previously (Grabstein et al. 1994).

### **2.2 Structure and expression**

The IL-15 gene consists of 9 exons and 8 introns. In humans it is located in chromosome 4q31. The mature IL-15 protein is 114 amino acids in length, and the final protein is coded by 4 exons and 3 introns. (Anderson et al. 1995.) It has two disulfide bonds at positions 42 Cys – 88 Cys and 35 Cys – 85 Cys (Grabstein et al. 1994).

At least in humans and mice, IL-15 has two isoforms. Alternative splicing creates two different mRNA variants. They differ in the signal peptide which is responsible for the transport of the protein. The signal peptide of IL-15LSP (long signal peptide) is 48 amino acids in length, and the signal peptide of IL-15SSP (short signal peptide) 21 amino acids. (Budagian et al. 2006.) The existence of these isoforms has physiological implications. IL-

IL-15LSP is secreted by the cell, whereas IL-15SSP is not secreted by the cell, but stored in the cytoplasm. Intracellular IL-15-SSP downregulates IL-15 gene transcription by localizing in the nucleus together with IL-15 Receptor  $\alpha$  (IL-15R $\alpha$ ). Thus, IL-15 production is autoregulated. (Nishimura et al. 2005.)

Even though many tissues express the IL-15 gene, the mRNA levels often do not correlate with protein levels. IL-15 protein expression is regulated on multiple levels in transcription and translation. (Pistilli & Quinn 2013.) Also, the presence of soluble IL-15R $\alpha$  in biological samples can make it difficult to detect free IL-15, because of the formation of receptor-ligand complexes between the two molecules (Budagian et al. 2006). This complex can mask the presence of IL-15 when measured, since many antibody preparations will not detect it (Pistilli & Quinn 2013). The translation phase has three major checkpoints. When all three are cleared, IL-15 translation increases 250-fold. (Bamford et al. 1998.) Based on the presence of these checkpoints, it seems that IL-15 mRNA can exist in translationally inactive pools. It is possible that large amounts of IL-15 protein could exert harmful effects on the cell. (Budagian et al. 2006.)

### 2.3 Signaling

*Shared receptor strains with IL-2.* IL-15 has shared bioactivities but no sequence homology with IL-2. This has important implications on signaling. The most common receptor for IL-15 consists of three subunits:  $\alpha$ ,  $\beta$  and  $\gamma$ . Receptor subunits  $\beta$  and  $\gamma$  are the same for IL-2 and IL-15 (named IL-2R $\beta$  and IL-2R $\gamma$ ). Only the  $\alpha$  subunit of the receptor is distinct between the two. (Giri et al. 1994.) This leads to some similarities in the signaling action caused by IL-2 and IL-15. However, it is clear that IL-2 and IL-15 exert many distinct responses. The signal pathway of IL-15 receptors depends on the receptor structure, which can be different in different tissues. (Budagian et al. 2006.) An overview of possible signaling pathways is presented in Figure 1.

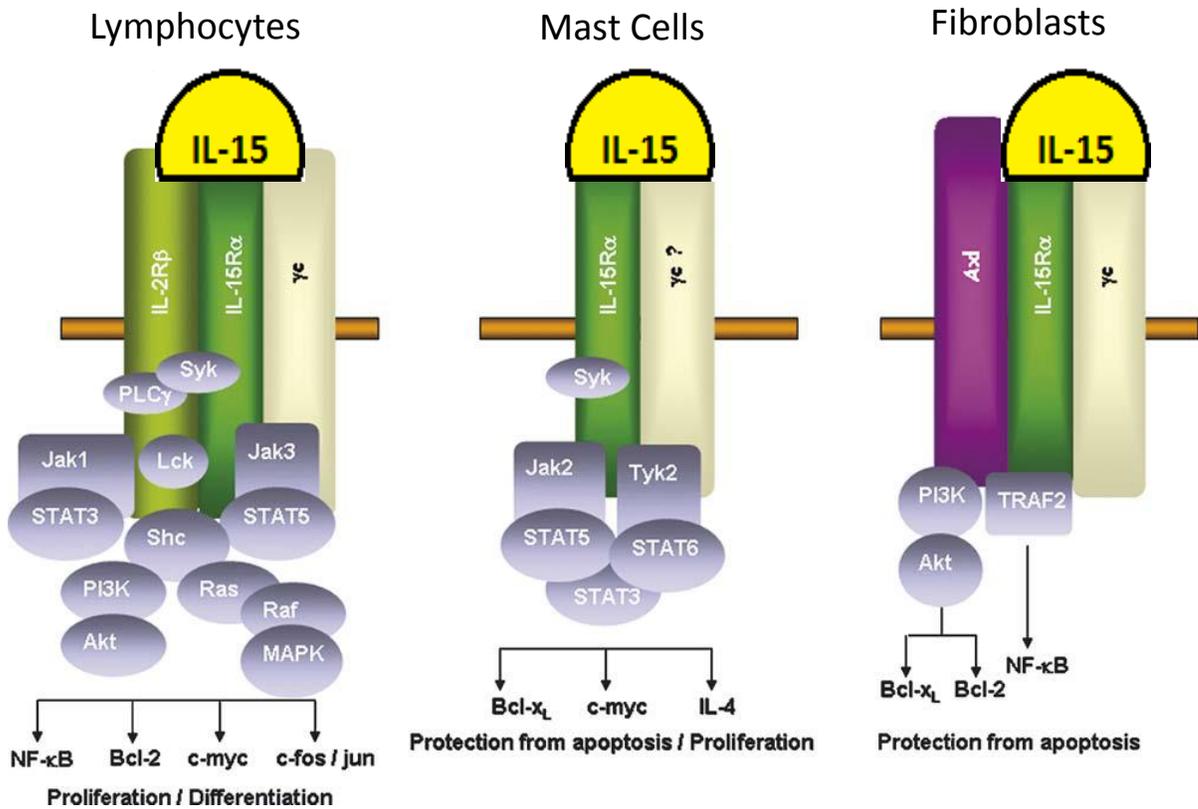


FIGURE 1. IL-15 signaling in different receptor types (Adapted from Budagian et al. 2006).

*Receptor components.* IL-15R $\alpha$  has an important role in the functional system of IL-15. It has high affinity for IL-15, even in the absence of the other main receptor subunits IL-2R $\beta$  and IL-2R $\gamma$  (Giri et al. 1994). As with IL-15, IL-15R $\alpha$  is expressed a variety of cells and tissues: at least T cells, B cells, NK cells, macrophages, thymic and bone marrow cells, brain, intestine, liver, skeletal muscle, lung, heart as well and kidneys. This makes interaction between different tissues theoretically possible via the mediation of IL-15. (Budagian et al. 2006.)

IL-15R $\alpha$  exists in free soluble form as well as cell membrane-bound form (Budagian et al. 2006). Soluble IL-15R $\alpha$  can be present in intracellular and extracellular compartments. It seems that when bound to free IL-15, its function is to stabilize the IL-15 protein and limit its bioavailability. (Bergamaschi et al. 2008.) It is also possible that soluble IL-R $\alpha$  interacts

with the membrane-bound IL-15 receptor complex to modulate IL-15 signaling (Budagian et al. 2006).

The other two receptor subunits (IL-2R $\beta$  and IL-2R $\gamma$ ) have lower affinity for IL-15, but they can nonetheless bind to free IL-15 without the presence of IL-15R $\alpha$  (Giri et al. 1994). Therefore it seems that tissues with an active IL-15R $\alpha$  component are more sensitive to circulating IL-15 compared to tissues with only IL-2R $\beta$  and IL-2R $\gamma$  subunits present (Crane et al. 2015). This can explain why pathogen-like immune system responses and muscle-derived IL-15 adaptations are not necessarily related despite having the same signal molecule. It is easy to imagine that local IL-15 concentrations mediating an inflammatory signal to neighboring cells presenting the IL-2R $\beta$  and IL-2R $\gamma$  receptor components can be much higher than systemic IL-15 concentrations mediating a signal to different tissues presenting a receptor with IL-15R $\alpha$ .

*Signaling pathways.* At least in lymphocytes and mast cells, the presence of IL-15 in the IL-15 receptor activates the JAK-STAT-pathway. In this process, the janus kinase (JAK) proteins are activated through cross-phosphorylation. The JAKs then phosphorylate signal transducers and activators of transcription (STAT proteins). The specific JAK and STAT proteins that are activated depend on the receptor structure. The signal pathway activated by the IL-15R $\alpha$  subunit involves JAK2, STAT3, STAT5 and STAT6. (Budagian et al. 2006.) IL-2R $\beta$  activates the JAK1-STAT1-pathway, and IL-2R $\gamma$  activates the JAK3-STAT5-pathway (Johnston et al. 1995).

IL-15 receptors of different types have also been shown to activate several other pathways. Tyrosine kinases of the Src family can be activated through phosphorylation. Other pathways that can be activated are the PI3-kinase, Akt, Bcl-2 anti-apoptotic protein, and the MAPK-pathways. IL-15 can also activate transcription factors NF- $\kappa$ B and AP-1, and affect c-myc (a regulator gene). The IL-15 receptor is in some tissues (such as fibroblasts) connected to receptor tyrosine kinase Axl, which is activated by the IL-15R $\alpha$  subunit. Overall, a large number of pathways are possible. Hence there is also potential for

interaction with other signaling networks, which are based on completely unrelated receptors. Such diversity in downstream signaling can have important biological meaning, because this allows the cell to integrate the effects of several receptor systems. (Budagian et al. 2006.) The signaling pathways for skeletal muscle under different physiologic conditions are not clear (Pistilli & Quinn 2013).

The signaling of IL-15 is not limited to soluble IL-15 in biological fluids. Juxtacrine signaling is possible through a mechanism where IL-15 is produced in the cell where it binds to IL-15R $\alpha$ . The receptor-ligand complex is then presented on the outside of the cell membrane, where it can interact with IL-2R $\beta$  and IL-2R $\gamma$  of neighboring cells, also called transpresentation. In addition, the complex consisting of IL-15 and IL-15R $\alpha$  can be released out of the cell. (Dubois et al. 2002.) In conclusion, IL-15 signaling is quite complex. There are several possible signal pathways which are different between tissues. IL-15 protein expression and availability is regulated on multiple levels. Hence the availability and action of IL-15 in each individual tissue depends on a large amount of factors. (Pistilli & Quinn 2013.)

## **2.4 Role in the immune system**

IL-15 was initially associated with the immune system. Together with many other cytokines, IL-15 is a mediator of inflammation. The inflammatory response is created when macrophages in a tissue become active and start secreting cytokines. Within the immune system, IL-15 has several documented roles, such as proliferation, differentiation and inhibition of apoptosis in several cell types. (Budagian et al. 2006.) When IL-15 was first discovered, it was shown to mimic the actions of IL-2, mediating a signal for T-lymphocyte proliferation and survival (Grabstein et al. 1994). IL-15 and IL-15 receptor biology in T cells and NK cells is well covered in the literature. For a review, see Budagian *et al.* (2006).

### 3 IL-15 IN SKELETAL MUSCLE

IL-15 is highly expressed in skeletal muscle. In fact, IL-15 is one of the most abundantly expressed cytokines at the mRNA level in human skeletal muscle. (Grabstein et al. 1994, Nieman et al. 2003). Skeletal muscle also expresses detectable levels of the IL-15 receptor subunits IL-15R $\alpha$ , IL-2R $\beta$  and IL-2R $\gamma$  (Pistilli, Siu & Alway 2007). Although much is known about IL-15 signaling pathways in other tissues, the exact role and signal pathways of muscle-derived IL-15, both within muscle and in other tissues is not clear. Possible signaling mechanisms are at least: 1) inhibition of TNF- $\alpha$ -based catabolic pathways, 2) direct influence on intracellular protein synthesis and degradation pathways, and 3) influence on muscle oxidative metabolism through pathways mediated by signals such as sirtuins, PPAR $\delta$  or PGC-1 $\alpha$ . (Pistilli & Quinn 2013.) Increases in the levels of TNF- $\alpha$  within skeletal muscle have been linked to aging (for a review, see Phillips & Leeuwenburgh (2005)). It is believed that preservation of muscle mass by IL-15 is carried out through inhibition of TNF- $\alpha$ -triggered apoptosis (Marzetti et al. 2009). This is possible via a NF- $\kappa$ B-activated pathway (Wang et al. 1998), which in turn has been shown to be affected by IL-15 signaling (Budagian et al. 2006).

#### 3.1 Physiology in skeletal muscle

*Anabolic effects from studies in vitro.* IL-15 has been shown to induce muscle hypertrophy *in vitro* using myotube (immature muscle fiber) cell cultures. Hypertrophy occurs due to both an increase in protein synthesis and a reduction in protein breakdown. (Quinn et al. 2002.) However, the nature of the *in vitro* anabolism is distinct from other anabolic factors such as insulin-like growth factor 1 (IGF-1). IL-15 does not normally affect myoblast differentiation, which IGF-1 mediated hypertrophy for example largely depends on. (Pistilli & Quinn 2013.) One important exception is the study by Quinn *et al.* (1997). They found that the addition of IL-15 caused differentiation of myoblasts into muscle cells in cell

cultures where the action of IGF-1 was repressed. Conditions with reduced concentrations of IGF-1 can arise naturally in catabolic states or with normal aging. (Quinn et al. 1997.)

*Effects on muscle atrophy.* The ability of IL-15 to cause anabolic effects in muscle cell cultures has not been fully replicated *in vivo* despite a number of attempts (Pistilli & Quinn 2013). However, it seems that IL-15 can still mediate protection against muscle atrophy. It has been shown that conditions linked to muscle atrophy such as aging (Quinn et al. 2010, Marzetti et al. 2009) and unloading (Pistilli et al. 2007) coincide with a change in muscle IL-15 signaling. Pistilli *et al.* (2007) found that hind limb suspension in rats and wing unloading in quail resulted in increased IL-15 mRNA expression. In unloading conditions, increasing the production of IL-15 would make sense as an attempt to preserve muscle. Direct evidence of counteraction of muscle atrophy was then observed in animal studies using experimental IL-15 administration (Pistilli & Quinn 2013). A summary of these studies is presented in Table 1.

TABLE 1. Studies with experimental IL-15 administration evaluating IL-15 action against muscle atrophy.

<b>Authors</b>	<b>Intervention</b>	<b>Findings</b>
Carbo <i>et al.</i> (2000), Figueras <i>et al.</i> (2004)	7 d IL-15 administration on cachectic rats	Muscle mass sparing, inhibition of TNF- $\alpha$ pathway
Harcourt <i>et al.</i> (2005)	4 w IL-15 administration on dystrophic (mdx) mice	Improved diaphragm strength and cross-sectional area
Pistilli & Alway (2008)	14 d IL-15 administration on healthy young and aged rats	No effect on muscle mass

Thus, IL-15 has anabolic effects on muscle only in cell cultures. IL-15 is able to prevent muscle atrophy in atrophic conditions, but in healthy animals it is not sufficient to cause anabolic effects. The discrepancy between *in vitro* and *in vivo* experiments is at least partly explained by the action of IL-15R $\alpha$  in both intracellular and extracellular compartments: soluble IL-15R $\alpha$  is able to affect the expression, translation and signaling of IL-15. It is possible that healthy animals also possess other mechanisms which can counterregulate IL-15 action. (Pistilli & Quinn 2013.) Based on these findings, IL-15 could aid in the treatment of muscle wasting in conditions such as aging, cancer or muscle dystrophy. This could be achieved either through administration of IL-15 or by influencing the IL-15 signaling pathways. (Quinn et al. 2002.)

*Regulation of oxidative properties.* Interestingly, some new findings were observed in transgenic mice overexpressing IL-15. In mice with skeletal muscle-specific oversecretion of IL-15, both slow and fast-twitch muscles increased their expression of slow troponin I and reduced their expression of fast troponin I. An increase was found in the expression of other oxidative status markers, such as SIRT1 and UCP2. These findings indicate that IL-15 drives the muscles towards a more oxidative phenotype. (Quinn et al. 2011.) Similarly, IL-15 knockout mice expressed less myosin heavy chain 1 mRNA, driving the muscle towards a less oxidative phenotype (Quinn et al. 2014). This has some consequences regarding the role of IL-15 in muscle. Slow-twitch fibers are smaller than fast-twitch fibers. Therefore a switch towards more oxidative fibers would lead to a reduction of muscle mass. In order to retain muscle mass, some hypertrophy is needed. Hence an anabolic effect of IL-15 on muscle could be masked by a general shift towards oxidative fibers. Also, slow-twitch fibers are more resistant to muscle atrophy. This could in part explain why IL-15 seems to mediate protection against atrophy in live animals. (Pistilli & Quinn 2013.)

*Role of IL-15R $\alpha$ .* Pistilli *et al.* (2011) studied the effects of IL-15R $\alpha$  knockout on mice. In their experiment, the knockout mice ran six times the distance in a running wheel when compared to control mice. Their fast muscles showed greater fatigue resistance and a more oxidative phenotype. The authors explained these results by a greatly increased effect of IL-

IL-15 signaling. Indeed, the IL-15R $\alpha$  knockout mice also showed greater IL-15 mRNA expression, which is explained by the absence of IL-15R $\alpha$  repression on IL-15 gene expression. In addition, IL-15R $\alpha$  knockout results in greater bioavailability of IL-15, since a portion of the total IL-15 is no longer bound to IL-15R $\alpha$ . (Pistilli et al. 2011.)

### 3.2 Interaction with other tissues

*Serum.* IL-15 is found in serum, both in soluble form and in a complex together with IL-15R $\alpha$ , which stabilizes IL-15 (Bergamaschi et al. 2012). Most of the circulating IL-15 is believed to originate from skeletal muscle because of its high expression of IL-15 mRNA (Quinn et al. 2005). A muscle-derived IL-15 signal can reach other tissues through the bloodstream.

*Adipose tissue.* The evidence that links muscle IL-15 production with adipose tissue dynamics is convincing. IL-15 inhibits adipocyte differentiation *in vitro* (Quinn 2008). Muscle-specific overexpression or experimental administration of IL-15 reduces fat mass in both lean and obese rodents, as has been shown in several studies (Quinn et al. 2009, Nielsen et al. 2008, Alvarez et al. 2002). Similarly, mice with a targeted deletion of IL-15 showed greatly increased fat mass (Barra et al. 2010). In humans, circulating levels of IL-15 have a negative correlation with obesity and abdominal fat (Nielsen et al. 2008). It has also been shown that polymorphisms in the human genes for IL-15 and IL-15R $\alpha$  are linked to adiposity and markers of metabolic syndrome (Pistilli et al. 2008, Di Renzo et al. 2006). However, it is interesting to find that adipocytes or preadipocytes produce very little or no IL-15 mRNA themselves. This means that the IL-15 affecting adipose tissue probably comes from other sources. Therefore it seems that IL-15 mediates a signal on the muscle-to-fat axis, modulating fat-lean body composition. (Quinn et al. 2005.)

A model for IL-15 mediated interaction between fat and muscle tissue was proposed by Carbo *et al.* (2001) (Figure 2). It is a two-way interaction. Muscle-derived IL-15 sends a signal to adipose tissue for reducing fat mass. Adipose tissue in turn produces certain

signals back to skeletal muscle, signaling muscle protein degradation. This proposed interaction is reciprocal, with fat and muscle tissue regulating each other. (Carbo et al. 2001.) This can explain, for example, why obese animals show a reduction in muscle protein content (Lanza-Jacoby & Kaplan 1984). The signal released from adipose tissue affecting skeletal muscle is carried out through TNF- $\alpha$ , leptin (Carbo et al. 2001), adiponectin and resistin (Quinn et al. 2005).

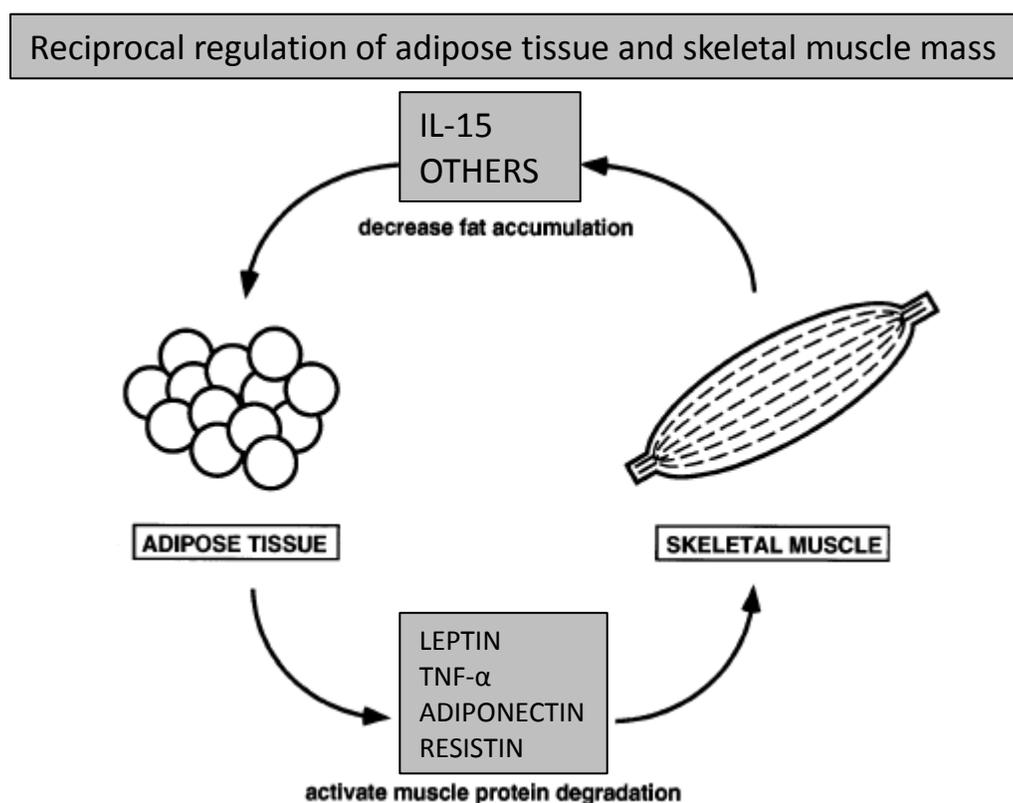


FIGURE 2. A proposed model for the interaction between muscle and fat as mediated by IL-15 (Adapted from Carbo et al. 2001).

*Bone.* Circulating IL-15 also affects other tissues. Quinn *et al.* (2009) studied mice that overexpressed the secretable isoform of IL-15 in skeletal muscle. Long-term oversecretion of IL-15 increased bone mineral density.

*Skin.* The results from Crane *et al.* (2015) suggest that muscle-derived IL-15 is connected to health benefits on the skin by promoting mitochondrial function. They showed that endurance exercise could attenuate age-associated changes in the skin, mediated through IL-15. They also showed that muscle AMPK mediates the signal in muscle. In their study, muscle-specific reduction in AMPK resulted in problems in skin structure similar to those with aging, with IL-15 as the mediator signal.

### 3.3 Effects of exercise

Some have tried to assess the effects of exercise on IL-15 in muscles and the bloodstream. However, the results are controversial and quite few in number to draw definite conclusions. Also, inaccurate measurement of IL-15 has been a problem in some of these studies.

*Acute effects on IL-15 mRNA and protein.* No change was found in muscle IL-15 mRNA in human marathon runners immediately after 3 hours of running (Nieman *et al.* 2003). 2 hours of strength training did not change muscle IL-15 mRNA, although an increase was found in all other measured cytokines: IL-6, IL-8, IL-1 $\beta$  and TNF- $\alpha$  (Nieman *et al.* 2004). Nielsen *et al.* (2007) measured muscle IL-15 mRNA, as well as muscle and plasma IL-15 protein content after a bout of resistance training at 6, 24 and 48 hours of recovery. At 24 hours of recovery they found a twofold increase in IL-15 mRNA. There were no changes in muscle or plasma IL-15 protein content. According to the authors, this can be explained by a translationally inactive mRNA pool, which means no final protein is produced.

Tamura *et al.* (2011) showed an increase in serum IL-15 protein after 30 minutes of treadmill running in humans. A significant increase was found 10 minutes post-exercise, and a significant decrease compared to the 10-minute mark was found post-exercise, 1 hour and 3 hours after the bout. Therefore it seems that serum content of IL-15 rapidly increases after exercise, but also quickly decreases towards baseline within 60 minutes. Other studies have also found an acute increase in serum IL-15 following endurance (Crane *et al.* 2015) and resistance exercise (Riechman *et al.* 2004). No change was found in serum IL-15 in

humans during and after 2.5 hours of running (Ostrowski et al. 1998). However, the authors drew the conclusion that IL-15 was not measured precisely enough. The discrepancy between no observed changes in IL-15 mRNA expression but acute increases in circulating IL-15 could be explained by changes in the action of IL-15R $\alpha$  (Pistilli & Quinn 2013).

*Long-term effects.* Few studies have been conducted looking at effects of long-term exercise. IL-15 was not differently secreted from muscle following 1 week of endurance (Roca-Rivada et al. 2012) or 10 weeks of resistance exercise training (Riechman et al. 2004). Interestingly, an acute increase in serum IL-15 was observed following exercise in physically active, but not in sedentary subjects (Crane et al. 2015).

*Role of IL-15R $\alpha$ .* Genetic variation in the IL-15R $\alpha$  gene can have influence on the training response. Two separate single nucleotide polymorphisms in the gene were associated with variation in hypertrophy in humans over a 10 week resistance training period (Riechman et al. 2004). This is somewhat confounding, since IL-15-mediated hypertrophy is normally not observed *in vivo*. The mechanisms underlying this result are not clear.

### **3.4 Fiber type characteristics**

Basal levels of IL-15 mRNA have been found to be twofold larger in the fast-twitch triceps compared to the slow-twitch soleus. However, there was no difference in IL-15 protein content between the muscles (Nielsen et al. 2007). It is important to keep in mind that IL-15 does have an effect on the fiber type itself, driving muscles towards a more oxidative phenotype (Quinn, Anderson et al. 2011).

### 3.5 Effects of aging

Serum and muscle IL-15 protein levels decrease in aging mice (Quinn et al. 2010). Similarly, intramuscular IL-15 protein levels decrease with aging in rats (Marzetti et al. 2009). IL-15 action has been shown to cause myoblast differentiation and hypertrophy when the levels of bioavailable IGF-1 protein decline, which happens with normal aging (Quinn et al. 1997). Therefore it seems possible that influencing the IL-15 pathways could be a therapeutic aid for some of the muscle wasting with age. However, no correlation was found between age and serum IL-15 in a study on 335 human subjects between the ages of 25 and 90 (Lamana et al. 2010). Pistilli *et al.* (2007) found that aging and unloading induced more profound increases on IL-15 mRNA in the slow-twitch soleus muscles compared to the fast-twitch plantaris muscles. Therefore it could be speculated that aging results in more profound changes on IL-15 action in slow muscles compared to fast muscles. In their study, aging actually caused an increase in muscle IL-15 mRNA. The mechanisms underlying their result are not clear, in contrast to the findings on protein level by other groups (Quinn et al. 2010, Marzetti et al. 2009). To summarize, there is some discrepancy in the literature, possibly arising from different types of subject populations and mRNA compared to protein measurements. Changes in IL-15 protein expression can be different to changes in the expression on mRNA level, as these often do not correlate (Pistilli & Quinn 2013).

## **4 RESEARCH QUESTIONS AND HYPOTHESES**

### **4.1 Research questions**

1. Is there a difference in muscle or serum IL-15 protein content (fast, mixed or slow skeletal muscle or serum) between the HCR and LCR rat strains?
2. Is there a difference in IL-15 protein content (fast, mixed or slow skeletal muscle or serum) between adult and old rats?
3. Is there a difference in IL-15 protein content (fast, mixed or slow skeletal muscle or serum) between rats with running wheel and rats without running wheel?

### **4.2 Hypotheses**

1. HCR rats have higher IL-15 protein levels. It has been shown that gastrocnemius IL-15 mRNA is higher in HCR rats compared to LCR rats (Kivela et al. 2010, unpublished finding).
2. Muscle and serum IL-15 protein levels decrease with age (Quinn et al. 2010). Despite somewhat confounding results in the literature, this is the most representative result for the hypothesis in the present thesis, as the authors measured IL-15 protein in rodents for both serum and muscle tissue. Changes are more prominent in slow muscles compared to fast muscles (Pistilli et al. 2007).
3. There is no change with running (Roca-Rivada et al. 2012, Riechman et al. 2004). Long-term physical activity does not influence chronic levels of IL-15-protein, but only acute responses (Crane et al. 2015).

## 5 MATERIALS AND METHODS

### 5.1 The animal model

Rats were used as research animals. This specific animal model was bred over several generations based on individual intrinsic running capacity. Generation 1 consisted of 168 rats, which were tested for aerobic capacity. This was done with a speed-ramped treadmill running test with 15° slope, initial velocity of 10m/min and a speed increase of 1m/min every 2 minutes. 26 of the best and worst performing rats were chosen to reproduce and constitute the 2 research groups (HCR = high capacity runner and LCR = low capacity runner). Over generations, the differences in intrinsic running capacity between the HCR and LCR groups became more pronounced. In generation 6 the mean difference in distance covered in the endurance test between the two groups was 171 %, and in generation 11 it was 347 %. (Koch et al. 2011.)

For this study we used 60 HCR/LCR female rats from generations 23 to 27. The research groups are presented in Table 2. Both the HCR and LCR groups were divided into 3 subgroups (n=10 per group): Adult rats (rats sacrificed at 9 months of age), old rats (rats sacrificed at 21 months of age) and running old rats (rats sacrificed at 21 months of age with continuous access to a running wheel). This yielded a total of 6 research groups: HCR, HCR old (HCR-o), HCR old runners (HCR-oR), LCR, LCR old (LCR-o), and LCR old runners (LCR-oR). The runner groups had access to a running wheel for the purpose of assessing voluntary running activity. The running wheel was presented to them at 9 months of age, so all 3 groups were homogenous up to this 9 month point. The running distance based on running wheel revolutions was measured with a computerized recording system to calculate monthly running distances for all the HCR-oR and LCR-oR rats. Running wheels were locked the evening before the day of sacrifice, meaning there is at least 12 hours between the last exercise session and taking samples.

Animals were housed in standard conditions: +22°C temperature, 50±10 % humidity and 12 hour day / night cycle. The animals had free access to food pellets (R35, Labfor, Stockholm, Sweden) and water.

TABLE 2. The research groups.

<b>Name</b>	<b>Intervention</b>	<b>Age at time of sacrifice</b>
<b>HCR</b>	No running wheel	9 mo
<b>HCR-o</b>	No running wheel	21 mo
<b>HCR-oR</b>	1 year of voluntary running with running wheel	21 mo
<b>LCR</b>	No running wheel	9 mo
<b>LCR-o</b>	No running wheel	21 mo
<b>LCR-oR</b>	1 year of voluntary running with running wheel	21 mo

## 5.2 Sample preparation

Three muscles were analyzed in this study: gastrocnemius, soleus and extensor digitorum longus (EDL) muscles. The muscles were obtained from the animals after necropsy at 9 or 21 months of age, snap frozen in liquid nitrogen and stored at -80°C. Frozen muscles were pulverized in liquid nitrogen and homogenized in an ice-cold buffer containing 20 mM HEPES (pH 7.4), 1 mM EDTA, 5 mM EGTA, 10 mM MgCl<sub>2</sub>, 100 mM β-

glycerophosphate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM DTT, 1 % NP-40, 0.2 % sodium deoxycholate and 3 % protease-phosphatase inhibitor cocktail (P78443, Pierce Biotechnology, Rockford, IL). The homogenate was then rotated for 30 minutes at +4°C and centrifuged for 10 minutes at 10000g and +4°C.

Total protein content of homogenates was determined using the bicinchonic acid protein assay (Pierce Biotechnology) with an automated KoneLab instrument (Thermo Scientific, Vantaa, Finland) for the purpose of equalizing protein concentrations for the SDS-PAGE procedure.

Blood samples were taken from the heart at the time of necropsy, held at room temperature for 30 min and centrifuged for 10 minutes at 2000g at room temperature. Plasma was then collected in separate tubes and stored at -80°C.

### **5.3 Blood analysis with ELISA**

IL-15 protein content in the blood samples was analyzed with enzyme-linked immunosorbent assay (ELISA). The SEA061Ra kit (Cloud-Clone Corp, Houston, TX) was used, which contains antibodies against rat IL-15. All samples were analyzed in duplicates and compared to a duplicate standard curve. Absorbance was measured at 450 nm with a microplate reader (Thermo Scientific).

### **5.4 Western blot antibody validation and analysis of muscles**

IL-15 protein content in muscles was analyzed by western blot. This was done with rat-specific IL-15 antibodies (1:1000 ab7215, Abcam, Cambridge, UK). Full-length rat IL-15 protein (ab49905, Abcam) was used as a positive control in all SDS-PAGE runs for the purpose of identifying the correct IL-15 signal.

A great deal of work was done to validate the protocol used in conjunction with this specific antibody. 5 % milk in TBS-T was tested as blocking agent, but this yielded almost no signal on the expected IL-15 site at 15-25 kDa, so the decision was made to use bovine serum albumin (BSA) for blocking instead. This was in accordance with the protocol on a user review on the Abcam website, where the same antibody had been used. Abcam customer support was contacted to aid with the validation, and they provided us with the positive control, recombinant IL-15 protein (ab49905). Figure 3 shows the signal from ab7215 on rat gastrocnemius tissue and the positive control, ab 49905. In an attempt to reduce background, Abcam provided us with another antibody (ab85010). The resulting signal from this antibody (1:1000) is shown in Figure 4. As can be seen, the signal was relatively weak on the expected IL-15 sites. Ab85010 is in fact an antibody against human IL-15, but was predicted to work on rat based on high sequence homology. As a result, ab7215 was chosen to be used despite the background. It is interesting to note that soluble IL-15R $\alpha$  protein is of size 42 kDa in humans. It is possible that the used antibodies can detect it. Two separate bands were chosen for analysis: the bands appearing at 13 kDa and 25 kDa. These bands corresponded with bands found in the well for positive control (ab 49905), as shown in Figure 3. The assumption was that these bands represent different IL-15 isoforms: IL-15SSP and IL-15LSP, which were expected to be 17 and 19 kDa of size for rats, respectively. It must be noted that of the two bands found at 13 kDa and 15 kDa in Figure 3, the lower is the one assumed to represent IL-15SSP.

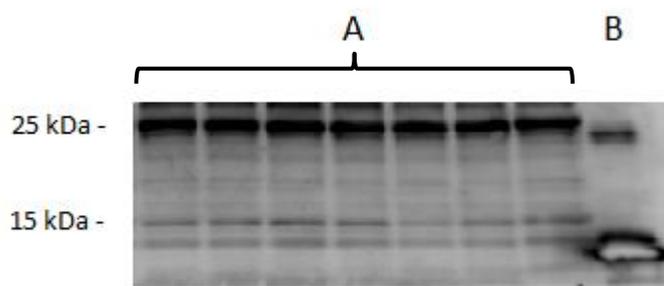


FIGURE 3. The signal of ab7215 in rat gastrocnemius muscle (A) and recombinant IL-15 (B).

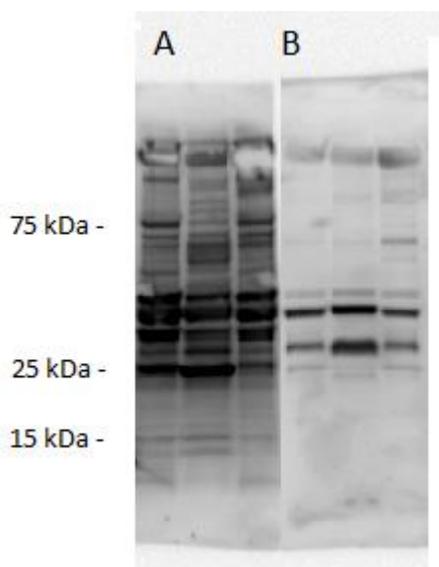


FIGURE 4. Western blots for rat gastrocnemius tissue with ab7215 (A) and ab85010 (B). IL-15 expected sites at 17 and 19 kDa in rat. IL-15R $\alpha$  expected site at 42 kDa in humans.

## 5.5 Western blot protocol

The chosen protocol was based on a user review on the abcam website for this specific antibody. Tissue lysate and sample buffer containing 30 µg of total protein was loaded in a 4 – 20 % gradient gel for SDS-PAGE (Bio-Rad Laboratories, Richmond, CA). Gel electrophoresis was done at +4°C with a voltage of 300 V in a Criterion cell (Bio-Rad Laboratories) for 25 – 30 min. Transfer of proteins from gel to a PVDF membrane was done at 300 mA for 2.5 h. The membrane was stained with ponceau-s for 5 min, which was then washed and photographed with a Chemidoc XRS device (Bio-Rad Laboratories). The membrane was blocked for 2 h in 5 % BSA in room temperature. The membrane was incubated overnight with primary antibodies (ab7215 with a dilution of 1:1000) at +4°C in 2.5 % BSA. Next morning the membrane was washed 4x5 min with TBS-T, followed by secondary antibody incubation for 1 h at room temperature in 5 % BSA. The membrane was then washed 5x5 min with TBS-T. Proteins were visualized with chemiluminescence (SuperSignal West Femto Maximum Sensitivity Substrate, Pierce Biotechnology). The signal was recorded with the ChemiDoc XRS device.

## 5.6 Western blot data analysis

The chemiluminescence images were analyzed with quantity one software (version 4.6.3, Bio-Rad Laboratories). All results are normalized to ponceau-s staining and tubulin protein content in the samples. Tubulin protein content was measured with anti-tubulin antibodies (T6199, Sigma-Aldrich, St. Louis, MO) after stripping the IL-15 antibodies. The ponceau-s and tubulin signals for each sample were compared to the mean ponceau-s and tubulin signals for each gel. The average of these two coefficients was used for normalization to both compare samples within gels and across different gels. For this to be a viable approach, the average ponceau-s and tubulin signals for each gel are assumed to only depend on the total amount of protein in the samples themselves, and otherwise be of similar intensity between gels. The structure of samples from different research groups was kept as equal as

possible between gels. It was also assumed that tubulin content should not significantly differ between the research groups. This was verified from the tubulin results.

## **5.7 Statistical analysis of results**

Statistical analyses for all variables were carried out using SPSS for Windows 20 statistical software (SPSS Inc., Chicago, IL). The Shapiro-Wilk test was used to investigate within group normality for a given parameter of interest. Levene's test was conducted to assess to homogeneity of the variance assumption. When the normality or equality of variance assumptions were not met, non-parametric tests were used, which was the case for all *post hoc* tests. The Mann-Whitney U-test was used for pairwise comparisons. Statistical significance was set at  $P < 0.05$ . Linear univariate analysis of variance was used to analyze the joint effect of rat strain, age and running. Running data was analyzed with the independent samples t-test.

## 6 RESULTS

IL-15 protein measurement data is presented for serum (measured with ELISA) and for the western blots performed on three muscle tissues: gastrocnemius, soleus and EDL. In the western blots, the selected antibody resulted in two different bands that corresponded with the positive control, assumed to represent IL-15SSP and IL-15LSP. Therefore, for each muscle, the results from two different signals are presented. The expected sizes for the isoforms were 17 kDa for IL-15SSP and 19 kDa for IL-15LSP. In the blots, the bands were found at 13 kDa and 25 kDa. The results are referred to as the 13 and 25 kDa isoforms of IL-15. Linear univariate analyses of variance for all IL-15 protein measurements are presented in Table 3.

### 6.1 Serum IL-15 protein

Serum IL-15 measurements from ELISA are presented in Figure 5. Adult rats from the LCR strain had significantly less IL-15 in serum compared to old LCR rats ( $P < 0.05$ ). A similar trend occurred in the comparison between adult and old HCR rats, but this was not statistically significant. There was a significant age effect (adult rats compared to old runners and non-runners,  $P < 0.05$ ).

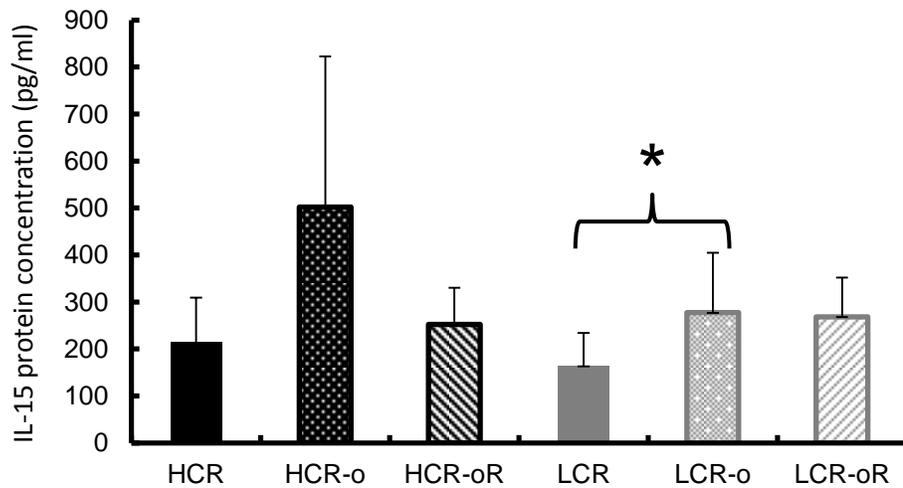


FIGURE 5. IL-15 protein content in serum measured with ELISA. Rats in the LCR-o group had statistically higher IL-15 concentrations compared to the LCR group ( $P < 0.05$ ). Values are expressed as mean  $\pm$  SD.

## 6.2 Muscle IL-15 protein

No statistically significant differences were observed between groups in the gastrocnemius in the 13 kDa band for IL-15 (Figure 6). For the 25 kDa band, there was a significant reduction in the LCR-oR group compared to both LCR and LCR-o ( $P < 0.05$ ) and HCR-oR ( $P < 0.01$ ). The trend for both isoforms was similar to the extent of LCR rats representing lower IL-15 protein content than HCR rats. Indeed, a significant strain effect was found, but only for the 25 kDa isoform ( $P < 0.05$ ).

In the soleus, there was a reduction in the IL-15 13 kDa isoform protein content in the HCR-oR compared to the HCR group ( $P < 0.01$ ) and in the LCR-oR compared to the LCR group ( $P < 0.01$ , Figure 7). There was also a significant age effect ( $P < 0.01$ ). No statistically significant differences were observed in the IL-15 25 kDa isoform.

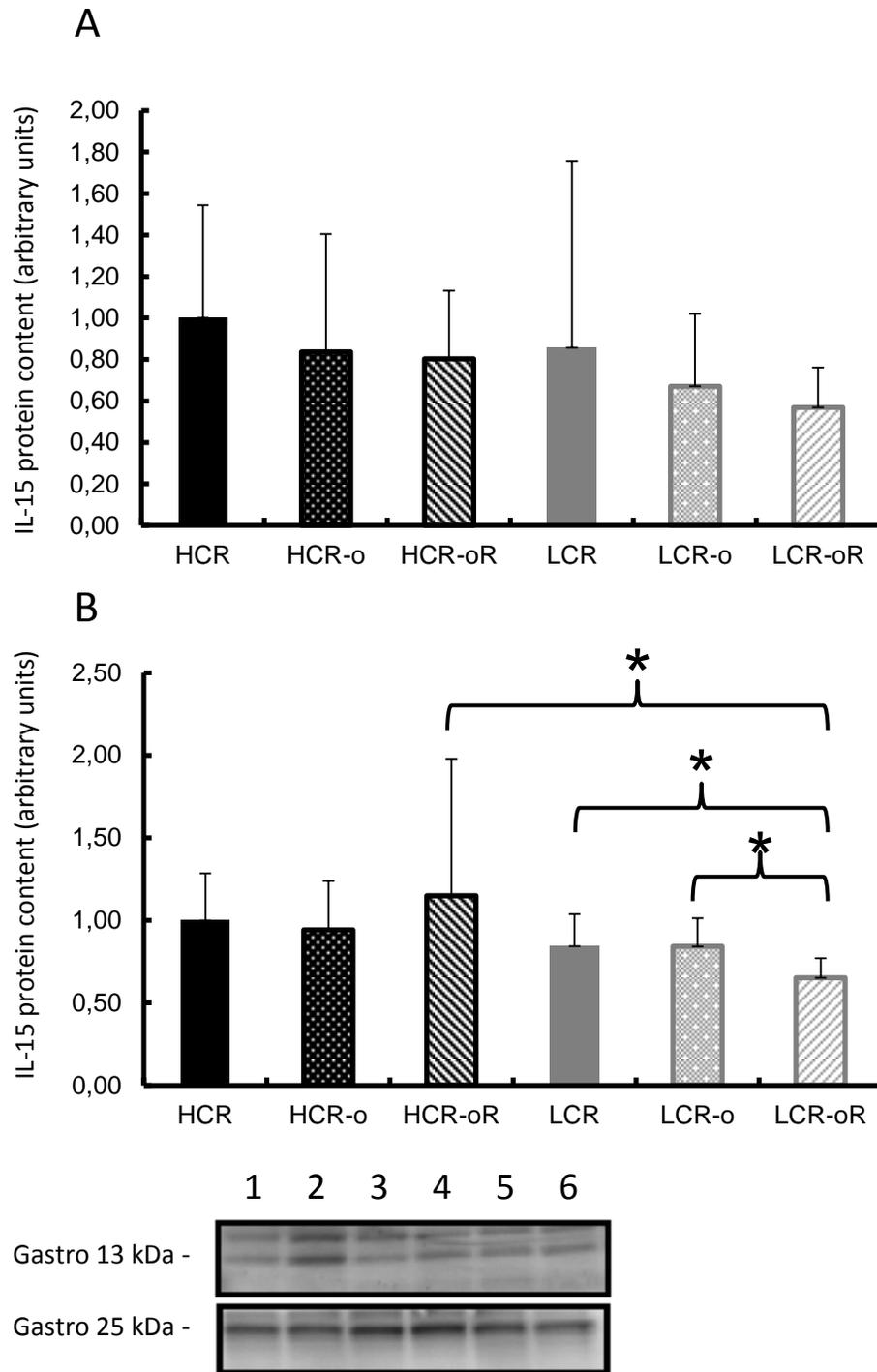


FIGURE 6. Gastrocnemius protein content for the two identified bands for IL-15 of sizes 13 (A) and 25 kDa (B). \* $P < 0.05$ , values are expressed as mean  $\pm$  SD. Representative western blot images for both measurements (C) with all research groups represented: LCR (1), HCR (2), LCR-o (3), HCR-o (4), LCR-oR (5) and HCR-oR (6).

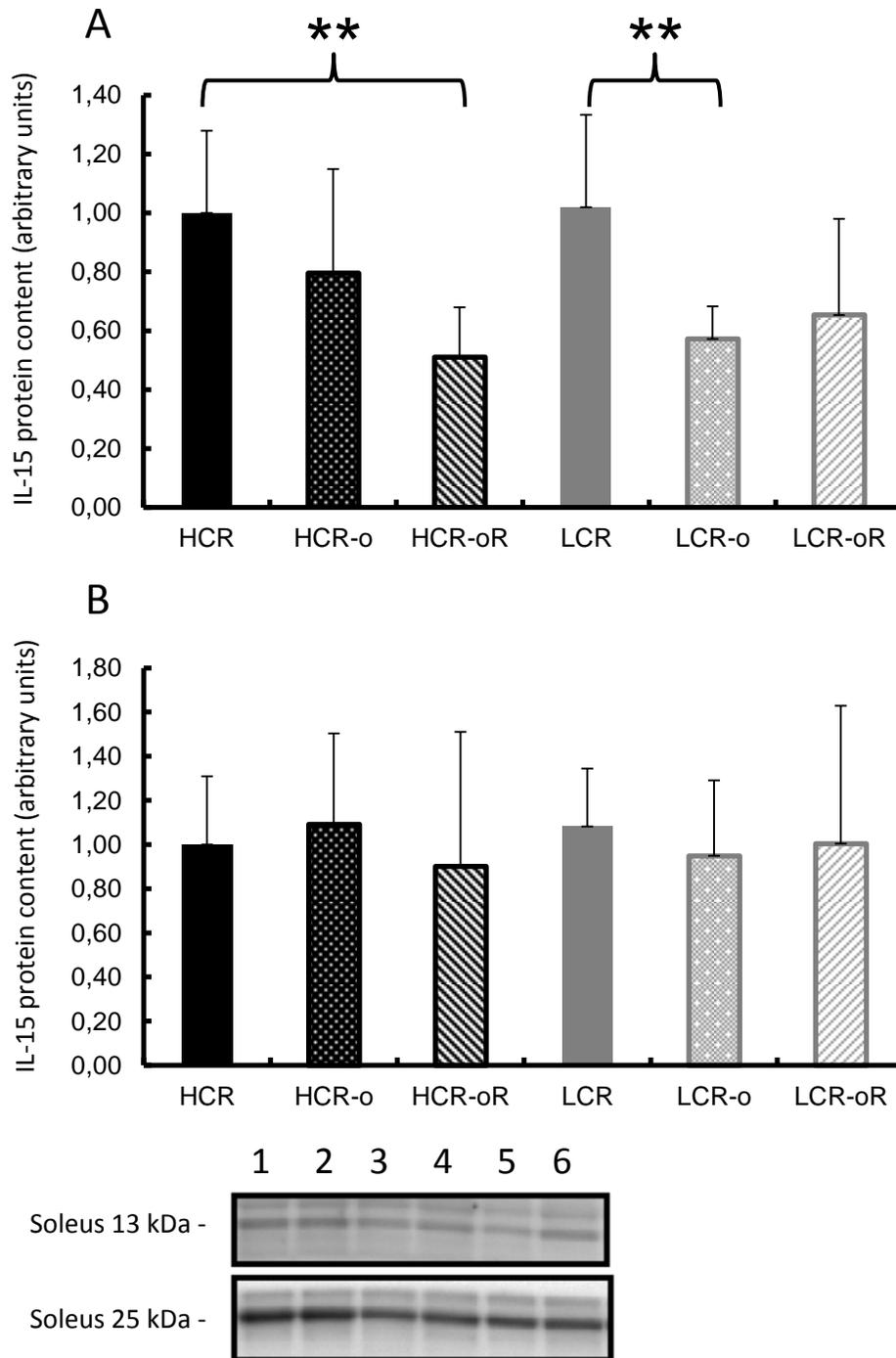


FIGURE 7. Soleus protein content for the two identified bands for IL-15 of sizes 13 (A) and 25 kDa (B).  $**P < 0.01$ , values are expressed as  $\text{mean} \pm \text{SD}$ . Representative western blot images for both measurements (C) with all research groups represented: LCR (1), HCR (2), LCR-o (3), HCR-o (4), LCR-oR (5) and HCR-oR (6).

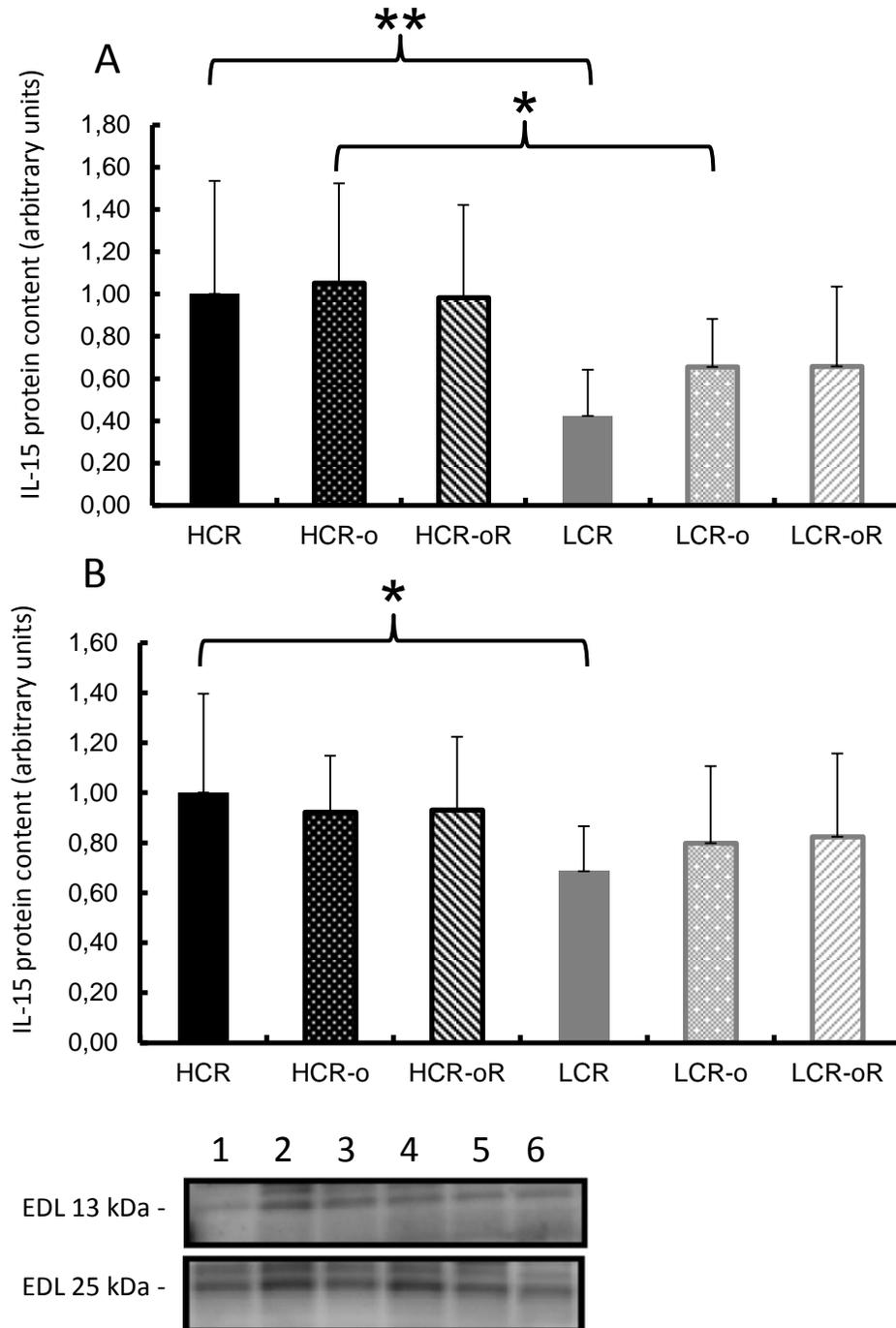


FIGURE 8. EDL protein content for the two identified bands for IL-15 of sizes 13 (A) and 25 kDa (B). \* $P < 0.05$ , \*\* $P < 0.01$ , values are expressed as mean  $\pm$  SD. Representative western blot images for both measurements (C) with all research groups represented: LCR (1), HCR (2), LCR-o (3), HCR-o (4), LCR-oR (5) and HCR-oR (6).

In the EDL muscle, a reduction in IL-15 protein content was observed in the LCR group compared to the HCR group for both the 13 kDa ( $P<0.01$ ) and the 25 kDa ( $P<0.05$ ) isoforms (Figure 8). In addition, a statistically significant reduction was also observed in the LCR-o group compared to the HCR-o group for the 13 kDa isoform of IL-15 ( $P<0.05$ , Figure 8). There was also a significant age effect for the 13 kDa isoform ( $P<0.001$ ).

TABLE 3. Linear univariate ANOVA results for IL-15 protein measurements. \* $P<0.05$ .

<b>Dependent variable</b>	Serum IL-15	Gastro 13 kDa	Gastro 25 kDa	Soleus 13 kDa	Soleus 25 kDa	EDL 13 kDa	EDL 25 kDa
<b>Strain</b>	0.332	0.355	0.014*	0.974	0.838	0.000*	0.157
<b>Age</b>	0.010*	0.353	0.979	0.005*	0.891	0.103	0.359
<b>Running</b>	0.082	0.622	0.716	0.520	0.761	0.578	0.782
<b>Strain x Age</b>	0.233	0.687	0.731	0.448	0.533	0.239	0.117
<b>Strain x Running</b>	0.105	0.789	0.362	0.069	0.245	0.898	0.965

### 6.3 Running data

Average daily running distances for each month are presented in Figure 9. HCR-oR rats had longer voluntary running distances in all time points compared to LCR-oR rats between the ages of 12 to 21 months.

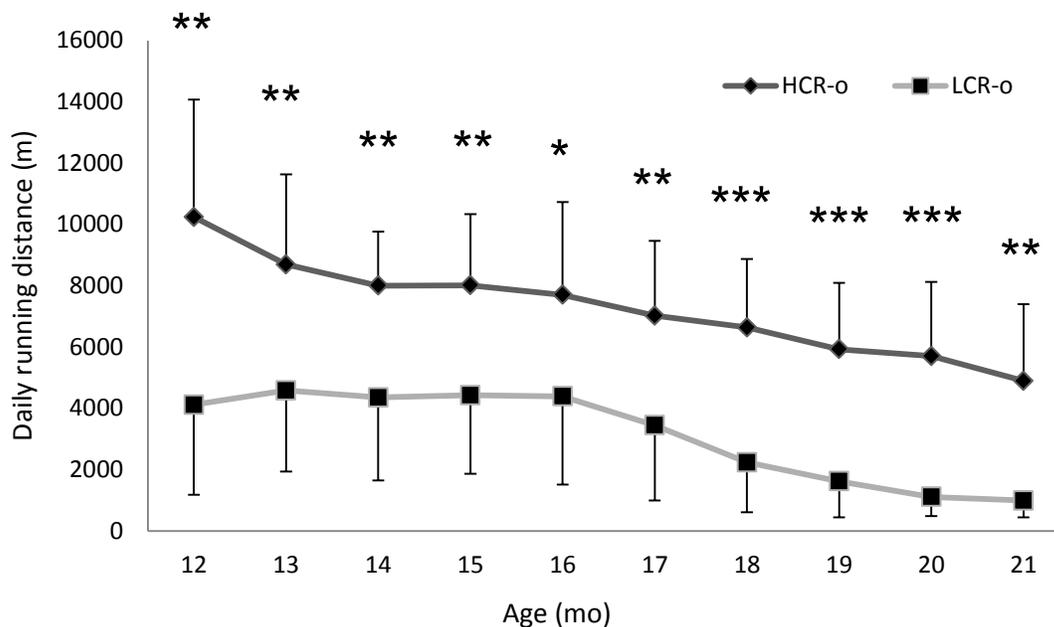


FIGURE 9. Daily average running distance for each month after the age of 12 months for old HCR and LCR rats with running wheel. \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001, values are expressed as mean ± SD.

## 7 DISCUSSION

The main finding of this study was that rats from the HCR strain showed elevated intramuscular IL-15 protein compared to LCR, as was hypothesized based on microarray mRNA data. This means that IL-15-mediated signaling can be responsible for explaining some of the differences found between HCR and LCR rats, both within muscle and in other tissues. These differences are related to both running capacity and general health, such as oxidative properties of different muscles or abdominal fat deposition. The main results and hypotheses are discussed below.

*HCR vs. LCR strain effects.* In the present study, strain differences among the 6 research groups in IL-15 protein content were found in the gastrocnemius and, more clearly, in the EDL. Univariate analysis revealed a significant strain effect for the gastrocnemius 25 kDa measurements and the EDL 13 kDa measurements, showing reduced IL-15 concentrations in the LCR compared to the HCR strain. The hypothesis in the present thesis was that HCR rats would have higher IL-15 protein levels, which is based on an unpublished finding in the study by Kivela *et al.* (2010). They observed an increase in gastrocnemius IL-15 mRNA in the HCR strain compared to the LCR strain ( $P < 0.001$ ) measured by microarray. The rats in their study were 9 months old females of generation 18 in the HCR/LCR strain development, so the conditions were very similar to the present study. Based on the present study, it seems that the increase in mRNA also results in increased IL-15 protein at least in the gastrocnemius and EDL. However, no strain effect was found in the oxidative type soleus muscle. The most pronounced changes were seen in the EDL, which is the fastest of the three muscles. It could therefore be speculated that the difference in IL-15 protein levels between HCR and LCR rats is more pronounced in faster limb muscles. Both slow and fast muscles show a wide spectrum of genetic differences between HCR and LCR rats, in rat gastrocnemius, soleus and EDL muscles (Kivela *et al.* 2010). The strain effects in the present study were found in different isoforms: 13 kDa (non-secreted IL-15) for the EDL

and 25 kDa (secreted IL-15) for the gastrocnemius, and it is difficult to say what relevance this spread has.

Contrary to the hypothesis, no strain effect was found in the serum. This can be attributed to a small number of measurements (the serum IL-15 measurement was only done on 5 rats per group, and standard deviations were quite large), the absence of increased IL-15 secretion from muscle, or to other mechanisms that regulate IL-15 bioavailability, such as the action of IL-15R $\alpha$  (Bergamaschi et al. 2008).

*Effects of aging.* An increase in serum IL-15 was observed in LCR-o rats compared to adult LCR rats. In the gastrocnemius, LCR-oR rats had a reduction in the IL-15 25 kDa isoform compared to LCR rats. No significant changes were found in the EDL with aging. In the soleus, old rats had a reduction in the IL-15 13 kDa isoform: HCR-oR rats compared to HCR rats and LCR-o rats compared to LCR rats. ANOVA also showed a significant age effect in the serum (increasing with age) and soleus 13 kDa isoform (decreasing with age). The hypothesis was that an increase in IL-15 would be seen with aging. While an aging-related increase was found in only two out of all group comparisons, it is still interesting that aging corresponded with an increase in serum IL-15 but a decrease in the soleus and gastrocnemius. The reasons for the result in serum is not clear, as Quinn *et al.* (2010) observed a decrease in serum IL-15 with aging in mice, measured with a bead-based quantitative immunoassay. However, a change has not always been found, as Lamana *et al.* (2010) found no correlation with age and serum IL-15 in 335 human subjects. Their results were measured with ELISA. For muscle, the results in the present thesis are consistent with the hypothesis, showing reduced amounts of IL-15 with aging. Muscle IL-15 has been shown to decrease with aging (Marzetti et al. 2009, Quinn et al. 2010), in both cases measured with a bead-based quantitative immunoassay. Pistilli *et al.* (2007) actually observed an increase in muscle IL-15 mRNA in aging rats, but this should not be automatically assumed to show in IL-15 protein expression, as the regulation between gene expression and protein availability for IL-15 in particular often causes mRNA to not correlate with protein (Pistilli & Quinn 2013).

It was also hypothesized that slow type muscle would show more prominent aging-related changes when compared to fast type muscle. This is consistent with the findings of the present study, as most changes were found in the most oxidative muscle tissue (soleus). The decrease in soleus IL-15 with aging was observed in the 13 kDa isoform. The 13 kDa isoform is thought to represent IL-15SSP, which is not secreted out of the cell. Therefore the observed reduction should not necessarily affect the serum IL-15 levels, possibly allowing it to increase because of other sources instead. It is possible that the IL-15 secretion system is different in muscles with different fiber type status. Net balance in the bloodstream is not dependent only on the soleus. It must also be kept in mind that measuring IL-15 protein concentration alone will not reveal the full picture. IL-15 trafficking on the muscle cell membrane and IL-15 bioavailability and stability both inside the muscle and in the bloodstream are unknown factors. They could be affected by molecules such as IL-15R $\alpha$ , which is found in all these compartments (Bergamaschi et al. 2008).

When interpreting the effects of aging in this study, it must be remembered that the HCR and LCR research groups were already adults. The samples of the adult rats were taken from 9 months old rats. The older rats (HCR-o, LCR-o, HCR-oR and LCR-oR) were 21 months old. The median lifespan in a standard environment is 24.0 months for LCR rats and 34.7 months for HCR rats (Koch et al. 2011). It is possible that some of the effects of aging have already occurred before the age of 9 months in rats, especially in the LCR strain.

*Effects of physical activity.* In the gastrocnemius, LCR-o rats showed an increase in the IL-15 25 kDa isoform compared to LCR-oR rats. No effect of running was found for any of the measured parameters. The hypothesis in the present thesis was that long-term physical exercise would cause no changes. The observed change in running only in LCR rats is interesting. It could be speculated that the LCR-o rats perform so little physical exercise that muscle degradation is increased, and that IL-15 production has been increased as a consequence, as proposed by Pistilli *et al.* (2007). The increase in IL-15 has a potential muscle mass sparing effect on these rats. It is not clear why the change was found in the

secreted (25 kDa) isoform, but not the 13 kDa isoform of IL-15, as it would make sense for this type of signal to affect the same muscle cell where it originates from. It is possible that the 25 kDa isoform is producing a signal to neighboring cells through transpresentation on its cell membrane (Dubois et al. 2002).

In previous studies, an acute effect has not been observed in mRNA measurements following endurance (Chan et al. 2004, Nieman et al. 2003) or resistance exercise (Nieman et al. 2004). When measuring serum protein levels, an increase in circulating IL-15 has been observed following both endurance (Tamura et al. 2011, Crane et al. 2015) and resistance exercise (Riechman et al. 2004). Based on the study by Tamura *et al.* (2011), it seems that IL-15 in the serum rapidly increases after exercise, but also quickly decreases towards baseline within 60 minutes. For long-term exercise, 1 week of endurance (Roca-Rivada et al. 2012) or 10 weeks of resistance exercise (Riechman et al. 2004) did not affect chronic IL-15 protein expression. However, it seems that physically active, but not sedentary humans show an acute increase in serum IL-15 following exercise (Crane et al. 2015). Therefore, it appears that an exercise-induced increase in circulating IL-15 protein is not based on increased gene expression, but post-transcriptional modulation. It also appears that in order to see an effect, sample collection after the last exercise session has to be correctly timed (within 30 minutes), and an increase should be seen in active subpopulations. In this regard, HCR-oR rats should conveniently represent the active subpopulation and a comparison can be made to LCR-oR rats (which are somewhat sedentary due to the strain effect on running habits, as can be seen from the data for daily running distances), HCR-o or LCR-o rats. In the present study, however, all rats were sacrificed more than 12 hours after locking the running wheels. While the optimal timing of blood samples has been studied, based on the literature it is not clear what the acute IL-15 protein expression profile is in skeletal muscles. It is possible that most IL-15 is produced and secreted into the bloodstream, and a peak concentration occurs before the corresponding peak in blood. As with the aging assessment, the modulation of IL-15 bioavailability and stability is an important consideration when interpreting IL-15 action. Thus, it is also possible that these factors are differently modulated between muscle and blood, and elevated IL-15

concentrations in muscle could be observed for a longer period of time following exercise. The best example of such a modulator seems to be IL-15R $\alpha$  (Bergamaschi et al. 2008). Nevertheless, no acute changes in muscle were seen in the present thesis, as more than 12 hours of rest is probably enough to let acute effects in both muscle and blood to return to baseline.

The methodology in the present thesis has not been used in many previous studies. The ELISA kit that was used has been used previously by one other group to measure rat IL-15 protein from the serum (Shamsi et al. 2014). It has been validated by the manufacturer, showing that cross-reactivity or interference with analogues to IL-15 is not significant. Coefficients of variation were reported to be <10 % intra-assay and <12 % inter-assay. For the western blot, the IL-15 antibody that was chosen has been used by at least one group. They wrote a review on the Abcam website on which the protocol in the present thesis was based, and reported the same 25 kDa band, but not the 13 kDa band. The reasoning for analyzing it in the present study was the correspondence with positive control. To summarize, a lack of publications using the chosen methodology in the present thesis leaves some uncertainty.

Limitations of this study include statistical quality and translating the findings into altered signaling pathways. Statistically significant differences between research groups were seen in only a small number of measurements. Because of this, it is difficult to accurately assess differences such as those between slow and fast muscles. The western blot data has some variance from a high background to signal ratio. A more optimized protocol and antibody choice could have yielded better results. The serum measurements with ELISA were done on only 5 rats per group because of economical limitations, and a bigger sample size could have given more significant results. Based on a measurement of IL-15 protein alone, definite conclusions about altered IL-15 signaling in different tissues cannot be drawn, since the outcome of different pathways also depends on other, potentially unknown factors. It must also be noted that all rats in this study were female. It is possible that male rats would have shown different patterns.

In conclusion, the present study showed that muscle IL-15 protein is elevated in HCR rats compared to LCR rats. This suggests that as a signal mediator both within muscle and to other tissues through the bloodstream, IL-15 action could be responsible for some of the differences observed between the HCR and LCR rat strains. Within skeletal muscle, IL-15 could affect oxidative status (Quinn et al. 2011) or muscle mass (Pistilli & Quinn 2013), which would help in explaining differences in running capacity. Health benefits with increased IL-15 protein levels could potentially be seen in visceral fat (Alvarez et al. 2002), bone (Quinn et al. 2009) and skin (Crane et al. 2015). If it can be shown that the observed differences in IL-15 protein have physiological outcomes, influencing the IL-15 signaling pathway is a potential therapeutic aid for individuals with an LCR-like genotype.

In future studies, it is important to assess the questions regarding IL-15 as a signal mediator in the bloodstream between HCR and LCR rat strains. This requires more in-depth investigation and knowledge about the regulation of IL-15 bioavailability and signaling pathways in muscle tissue, the bloodstream and peripheral target tissues. This includes, but is not necessarily limited to examining downstream signaling of IL-15 as well as the action of IL-15R $\alpha$ , which seems to be important in the regulation of IL-15 signaling (Bergamaschi et al. 2008). In addition, it is possible that some of the strain effects are seen acutely following exercise. Future studies should assess this aspect and take post-exercise samples before the 1 hour mark where serum IL-15 returns to baseline.

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