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Interleukin 1-beta Decreases Myoblast Fusion in vitro

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Interleukin 1-beta decreases myoblast fusion *in vitro*

Bethany Sullivan

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2015

Abstract

During times of stress, systemic pro-inflammatory cytokine levels are increased, which may prevent optimal growth and development of muscle and/or induce muscle atrophy. Pro-inflammatory cytokines can induce negative responses in muscle by altering the balance of protein synthesis and degradation in established myofibers, or by influencing the proliferation and differentiation of myoblasts. Interleukin-1 beta (IL-1 β) is a pro-inflammatory cytokine involved in stress and disease responses, but little is known about how IL-1 β affects myoblast function. We hypothesized that IL-1 β would decrease myoblast proliferation and/or differentiation. To test this hypothesis, C2C12 mouse myoblasts were treated with 0.1 ng/mL or 1.0 ng/mL of IL-1 β , or carrier only (control). To determine proliferation rate, myoblasts were plated at 2.6×10^3 cells/cm² and cultured for 48 h in the presence or absence of IL-1 β . Cells were pulsed with bromodeoxyuridine (BrdU), fixed, and immunostained. The number of BrdU positive cells was quantified as a percent of total nuclei (identified by Hoescht 33342). To determine if IL-1 β affected fusion, myoblasts were plated at 2.0×10^4 cells/cm² in growth media for 48 h at which time media was changed into differentiation media supplemented with 0.1 ng/mL or 1.0 ng/mL of IL-1 β , or carrier only. Cells were immunostained with myosin heavy chain (MyHC) and Hoescht 33342. Fusion index was determined by quantifying the number of nuclei within multinucleated myotubes divided by total nuclei. Finally, to determine the effect of IL-1 β on myotube size, myoblasts were cultured for 48 h in growth media and 48 h in differentiation media. Cells were cultured for an additional 48 h in the presence or absence of IL-1 β , fixed and immunostained for MyHC. Myotube diameter and fusion index were quantified. All data was analyzed using ANOVA in GraphPad Prism followed by Tukey's test for multiple comparisons. There were no significant effects of IL-1 β on proliferation or fiber diameter ($P \geq 0.05$). However, fusion was decreased 13.5% in myoblasts treated with 1.0 ng/mL

of IL-1 β ($P \leq 0.05$). In conclusion, IL-1 β decreases myoblast fusion, but does not affect proliferation or fiber diameter. These results suggest that IL-1 β may contribute to poor muscle growth by decreasing fusion of myoblasts into existing myofibers, preventing optimal hypertrophy.

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Review of Literature

Introduction

The effects of pro- and anti-inflammatory cytokines are affected by four variables: the timing of cytokine release, the other hormones present when the cytokines are released, the number of available receptors on the cell, and tissue responsiveness to each cytokine (Opal and DePalo, 1999). However, pro- and anti-inflammatory cytokines do not work independently; they interact with each other creating a coordinated level of control. Further, anti-inflammatory cytokines influence the secretion of pro-inflammatory cytokines throughout the body (Barton et al., 1996). There are distinctions between the mechanisms of each different cytokine, in that pro-inflammatory cytokines inhibit the immune response and anti-inflammatory cytokines repress the immune response. Interleukins (IL) are a subset of cytokines secreted from leukocytes with either pro- or anti-inflammatory characteristics (Dinarello and Mier, 1986). For example, IL-1 and tumor necrosis factor (TNF)- α are examples of pro-inflammatory interleukins that can induce negative responses in muscle by altering the balance of protein synthesis and degradation or by influencing the proliferation and differentiation of myoblasts (Spate and Schulze, 2004). Expression of IL-1 and TNF α is increased during times of stress such as changes in the environment, disease, and transportation, and this can prevent optimal growth and development of muscle. Interleukins have a wide variety of functions within the body and can induce protein degradation, stimulate myoblast proliferation, and create inflammation (Spate and Schulze, 2004). Interleukin-1 β is a pro-inflammatory interleukin involved with stress and disease responses and is responsible, at least in part, for detrimental effects such as inflammation and muscle atrophy in chronic diseases like cancer, chronic heart failure, and sepsis (Spate and Schulze, 2004).

Pro-inflammatory cytokines

Pro-inflammatory cytokines are molecules that prompt and facilitate local catabolic reactions which break down molecules and release energy, and this process can lead to muscle atrophy and the breakdown of metabolic materials (Spate and Schulze, 2004). These pro-inflammatory cytokines can also act as local and/or systemic markers of inflammation because the abnormalities created by these cytokines can be measured, and subsequent inflammation may affect the entire body or a specific tissue (Spate and Schulze, 2004). For example, chronic heart disease is associated with metabolic abnormalities which can lead to an increase in local concentrations of pro-inflammatory cytokines due to stress, and this may ultimately lead to skeletal muscle damage (Drexler et al., 1992). The metabolic abnormalities due to chronic disease and increase in pro-inflammatory cytokines cause a decrease in muscle oxidative capacity which leads to reduced muscle function (Drexler et al., 1992). It is not explicitly known how these pro-inflammatory cytokines cause detrimental changes locally within the muscle, but the interactions eventually lead to muscle atrophy (Spate and Schulze, 2004). Systemic concentrations of these cytokines can also affect the total body during periods of chronic disease. Although local and systemic concentrations may both be present in the same disease, having elevated local concentrations of pro-inflammatory cytokines does not necessarily indicate there will be elevated concentrations systemically as well (Spate and Schulze, 2004).

Interleukins

Interleukins, a group of pro- and anti-inflammatory cytokines, are products of macrophages or T cells, and induce cell growth, differentiation, and activation of other T cells (Mizel, 1989). Many different cell types, including macrophages, T cells, and cells within the

bone marrow, produce a wide variety of ILs (Mizel, 1989). Interleukins have various functions in the body and the potential to create a positive or negative effect on the tissue (Dinarello and Mier, 1986). For example, IL-1 and IL-1 β are pro-inflammatory, IL-10 is anti-inflammatory, and IL-6 has both pro- and anti-inflammatory characteristics (Dinarello and Mier, 1986).

Interleukin-10 inhibits the inflammatory response and promotes healing in the body. In men challenged with endotoxin, pre- or post-injection with IL-10 decreased the severity of symptoms caused by the LPS endotoxin (Pajkrt et al., 1997). This decrease in symptoms validates that IL-10 decreases the body's response, preventing negative effects, and further research could potentially show this is due to a decrease in immune response. Interleukin-10 works together with specific receptors to influence the immune system, thus achieving a specific response within the cell that will act on the entire body (Opal and DePalo, 1999).

Interleukin-6 is an example of an interleukin that has both pro- and anti-inflammatory characteristics. Barton et al. (1996) conducted an experiment that showed that IL-6 can have anti-inflammatory characteristics by injecting mice with d-galactosamine, causing systemic shock and ultimately the production of IL-6 within the body. Pretreatment with IL-6 antibodies, inhibiting IL-6, increased mortality, while a pretreatment with IL-6 itself decreased mortality. This showed that if IL-6 is present before disease consumes the body, there will be decreased mortality. The effects of IL-6 on skeletal muscle was also studied by Kim et al. (2004), who suggested that IL-6 has pro-inflammatory characteristics and causes a significant impairment of insulin action in skeletal muscle. They found there was a decrease in whole body insulin resistance in mice infused with an injection of 0.5 μ g of IL-6, which decreased the amount of glucose the skeletal muscle took up. Therefore, this decrease in glucose uptake caused significant impairment in the skeletal muscle such as a decrease in muscle glucose metabolism.

However, it has also been shown that this cytokine can do the opposite to skeletal muscle. Meng et al. (2014) showed that IL-6 is one of the factors that modulate muscle regeneration. RNF13 knockout mice had accelerated muscle regeneration which could be due to increase production of IL-4/IL-6. Therefore, they concluded that IL-6 facilitates satellite cell proliferation and potential muscle regeneration after injury.

IL-1

Interleukin-1 is a pro-inflammatory cytokine, produced by phagocytic cells such as blood monocytes and phagocytic cells that line organs (Dinarello and Mier, 1986). Cytokines produced by these cells elicit systemic effects on organ systems throughout the body, beyond the tissue from which they are produced. However, IL-1 can also have a local effect when it is produced from specialized cells like B cells and large granular lymphocytes (Dinarello and Mier, 1986). In patients infected with a localized bacterial infection, monocytes and macrophages within the tissue became active and released IL-1, resulting in pathological changes including inflammation and fever (Dinarello and Mier, 1986). Additionally, inflammation in pigs resulted in the production of IL-1 which stimulated the production of prostaglandin E₂ (PGE₂) from fibroblasts and induced fever and inflammation throughout the body (Saklatvala et al., 1984). Interleukin-1 β is a form of IL-1 which produces catabolic effects on protein metabolism (Spate and Schulze, 2004). This specific cytokine can trigger the local expression of nuclear factor kappa B (NF- κ B), a transcription factor resulting in the production of pro-inflammatory cytokines, creating negative effects such as inflammation and reduction of muscle growth (Spate and Schulze, 2004).

Increased concentrations of these pro-inflammatory cytokines, especially IL-1 β , cause detrimental effects in many chronic diseases including cancer, chronic heart failure, diabetes mellitus, and sepsis (Spate and Schulze, 2004). For example, during chronic heart failure, there is an increase in IL-1 β and a decrease in local expression of IGF-I (Schulze et al., 2003). In rats with myocardial infarctions, pro-inflammatory cytokines induced muscle atrophy, which was associated with a decrease in IGF-I production and an increase in IL-1 β production from skeletal muscle cells. Due to this increase in IL-1 β and decrease in concentration of IGF-I, there was a decrease in muscle cross-sectional area, which was expected because IGF-I plays a major role in the growth and differentiation of cells and supporting the hypothesis that IL-1 and IL-1 β cause severe detrimental effects to muscle strength and to the body in periods of chronic disease.

Despite having opposite effects, both pro- and anti-inflammatory cytokines are interrelated and can create a coordinated level of control. A specific anti-inflammatory cytokine can inhibit the function of a pro-inflammatory cytokine by acting similarly to a pro-inflammatory cytokine. Specific anti-inflammatory cytokines are able to bind to the same receptors as the corresponding pro-inflammatory cytokine, but since they bind with even greater affinity, the pro-inflammatory cytokine cannot bind, and thus, cannot cause a negative effect (Opal and DePalo, 1999). For example, IL-1 receptor antagonist (IL-1ra) is a protein that is capable of inhibiting IL-1 and IL-1 β because it competitively binds to the same receptor as IL-1 and IL-1 β (Opal and DePalo, 1999). However, the newly bound IL-1ra fails to send signals throughout the cell because it is not specific to the receptor and therefore, does not activate the cell. The accessory protein IL-1 will not be produced and the negative effects on the immune system will be inhibited, creating less of an effect on the body during a diseased state. Therefore, Opal and Depalo (1999) hypothesized that this anti-inflammatory cytokine has the ability to act as an

inhibitory factor to pro-inflammatory cytokines and could be an important factor to study the effects and treatments for chronic disease. Although IL-1 and IL-1 β create negative effects on the body, IL-1ra can decrease the production and lessen the effect on the body by mimicking the pro-inflammatory cytokine, binding to the receptor, and reducing the amount of receptors available for IL-1 and IL-1 β .

Pathway of IL-1 β secretion

When the innate immune system senses invasion by different bacteria or infection, the immune system is activated, starting the cascade of effects to produce pro-inflammatory cytokines and promoting the inflammatory response (Weber et al., 2010b). First, the immune system activates the NACHT, a leucine-rich repeat (LRR) and pyrin domain (PYD) -containing protein 3 (NALP3) inflammasome pathway which plays a critical role in acute and chronic inflammation and enhances the production of IL-1 β during different diseases. A three protein complex, the NALP3-inflammasome's primary role is to increase inflammation. One of the proteins that make up NALP3-inflammasome is caspase-1, which, once activated, processes the precursor of IL-1 β , regulating the release of its mature form. If caspase-1 is mutated, there are systemic increases in IL-1 β because caspase-1 cannot regulate the concentrations of IL-1 β in the body.

Interleukin-1 β is primarily produced by macrophages and monocytes, cells within the innate immune system, and it can also be produced by epithelial cells, endothelial cells, and fibroblasts (Weber et al., 2010a). This pro-inflammatory cytokine works in either a paracrine manner, via cell to cell communication, or systemically through a protein secretion pathway. Interleukin-1 β works as a positive feedback promoter, inducing the expression of itself when

concentrations rise within the body. IL-1 β binds to the IL-1 receptor creating a ligand-induced conformational change within the receptor. This facilitates recruitment of an accessory protein which ultimately leads to the activation of transcription factors, nuclear factor kappaB (NF- κ B) and an activator protein. It also activates the c-Jun N-terminal kinase (JNK) pathway as well as the nuclear p38 pathway. The JNK then phosphorylates proteins that are part of the activator protein which ultimately increases the expression of IL-1, IL-6, and IL-8. Nuclear p38 phosphorylates splicing regulatory proteins, which destabilizes the mRNA to control the production of IL-1 regulated transcripts, decreasing expression. Also, the binding of IL-1 β to the IL-1 receptor induces phosphorylation of heat shock proteins which are then used to induce IL-1 expression. In all, this binding increases the production of other pro-inflammatory cytokines as well as the regulation and production of IL-1 β .

Effects of IL-1 β on skeletal muscle

Muscle inflammation is a major symptom of many diseases and can create lasting and detrimental effects on the body. Skeletal muscle inflammation can be caused by many different cytokines, including IL-1 β . In animals with cancer, levels of IL-1 β are increased in the blood and this is linked with muscle atrophy (Spate and Schulze, 2004). Additionally, IL-1 β is associated with increased levels of myogenic transcription factors and an increase in the expression of p38 kinase which resulted in early myogenesis and differentiation. Fibronectin and integrin- β 1, which modify extracellular matrix assembly and signaling, were also increased in tumor bearing animals. Thus, due to a release of these factors, there was an early activation of satellite cells and an increase in inflammatory cells that had migrated into the skeletal muscle. Further, tumor bearing animals had decreased IGF binding protein 4 and 6 and an increase in

IGF binding protein 5, which could potentially alter the concentrations of IGF within the blood. Insulin-like growth factor is a critical hormone that is needed for skeletal muscle to proliferate and differentiate, resulting in muscle growth. Additionally, in the presence of IGF-1, a low concentration of IL-1 β significantly suppressed protein synthesis, myogenin expression and myoblast differentiation. It also impaired IGF-1 action within the muscle which decreased the ability of IGF-1 to promote protein synthesis.

Additionally, Grundtman et al. (2007) found there was an increase in IL-1 β in the muscle in patients with polymyositis and dermatomyositis, inflammatory diseases that are associated with muscle weakness and muscle fiber necrosis. In the muscle tissue from patients with polymyositis, there is an increase in pro-inflammatory cytokines, especially IL-1 β . This pro-inflammatory cytokine was mainly expressed by macrophages and fibroblasts within the muscle. Muscle wasting could be due to the presence of IL-1 β and the other pro-inflammatory cytokines involved with the disease, which may play a major role in the pathogenesis of polymyositis.

Grabiec et al. (2013) also conducted a study that determined the effects of IL-1 β on early myogenesis of mouse C2C12 cells. A three day treatment of IL-1 β increased fusion index and the rate of protein synthesis as well as levels of myogenin and major histocompatibility complex (MHC). Grabiec et al. concluded that IL-1 β activity was limited to the onset of myoblast fusion and increased the phosphorylation and expression of p38 kinase, leading to activation of essential pathways for myogenic differentiation.

Further, psychosocial stress within the body, stress resulting from a perceived threat, can lead to altered immune function and possibly development of psychological disorders. In mice experiencing repeated social defeats, there was an increase in IL-1 β concentrations (Wohleb et

al., 2001). Anxiety like behavior was associated with activation of the IL-1 receptor. Therefore, stress and psychological disorders, such as anxiety and depression, may be related to an increase concentration of IL-1 β which can lead to muscle wasting or necrosis, as shown by previous studies.

Conclusion

IL-1 β is a pro-inflammatory cytokine that is present during inflammation and hinders the growth of skeletal muscle. Stress and anxiety increase the concentrations of IL-1 β , however how IL-1 β affects myoblast function is not well known. The presence of IL-1 β may inhibit myogenesis by altering myoblast proliferation and/or differentiation, decreasing muscle growth. Therefore, the objective of this study was to determine how IL-1 β affects proliferation, differentiation, and myotube diameter. We hypothesized that IL-1 β would decrease myoblast proliferation and/or differentiation.

Materials and Methods

Cell Culture

C2C12 myoblasts were cultured in growth media (high glucose Dulbecco's Modified Eagle Medium [DMEM], 10% Fetal Bovine Serum [FBS], 1% penicillin/streptomycin, and 0.2% gentomycin) at 37° C in 5% CO₂ on gelatin coated culture dishes. Cells were thawed and cultured for at least one passage before experiments were started. Cells were passaged when they were approximately 70% confluent. Cell number was determined using a hemocytometer and cells were plated at 1×10^5 cells/cm² to start the next passage.

Proliferation

To evaluate the effect of IL-1 β on proliferation, C2C12 myoblasts were plated at 2.6×10^3 cells/cm² on a 12-well culture dish and cultured for 48 hours in the presence of 0.1 ng/mL IL-1 β , 1.0 ng/mL IL-1 β or carrier only (control). Triplicate wells were treated for each treatment and the experiment was replicated three times. Cells were pulsed with eoxymidine (EdU) using the Click-iT EdU Imaging Kit (Invitrogen). Cells were fixed in 4% formaldehyde in PBS for 15 minutes and immunostained following the manufacturer's protocol. Nuclei were visualized using Hoeschst 33342. Images were obtained using the Zeiss Observer Z1 Microscope, taking four images per well for a total of 12 images per treatment. The number of EdU positive cells was quantified and is presented as a percent of total nuclei.

Myoblast fusion

To evaluate the effect of IL-1 β on myoblast fusion, C2C12 myoblasts were plated at 2.0×10^4 cells/cm² on a 12-well culture dish in growth media for 48 hours. Media was changed to

differentiation media (2% horse serum [HS], 1% penicillin/streptomycin, 0.2% gentomycin in low glucose DMEM) supplemented with 0.1 ng/mL or 1.0 ng/mL IL-1 β , or carrier only for an additional 48 hours. Cells were fixed using 4% formaldehyde in PBS for 15 minutes and placed in blocking solution (Superblock containing 5% HS, 5% bovine serum albumin [BSA]) for 40 minutes. The cells were washed once with PBS and incubated in anti-myosin heavy chain (MyHC, Developmental Studies Hybridoma Bank, Iowa City, IA) supernatant for 1 hour at room temperature. The cells were washed with PBS three times and incubated with AlexFluor488 goat α -mouse secondary antibody (1:250) and Hoechst 33342 (1:500) in blocking solution for one hour in the dark at room temperature. Following extensive washes with PBS, cells were imaged using the Zeiss Observer Z1 microscope. The fusion index was determined by quantifying the number of nuclei within multinucleated MyHC-expressing myotubes divided by total nuclei.

Myotube diameter

To evaluate the effect of IL-1 β on myotube diameter, C2C12 myoblasts were plated at 2.0×10^4 cells/cm² on 12-well plates in growth media for 48 hours until confluent. Media was changed to differentiation media for 48 hours to allow formation of myotubes. Myotubes were cultured for an additional 48 hours in differentiation media supplemented with 0.1 ng/mL or 1.0 ng/mL IL-1 β , or carrier only. Cells were fixed and immunostained for MyHC as described above. Myotube diameter was measured at three distinct places on each MyHC-expressing multinucleated fiber and used to calculate an average diameter for each myotube.

Statistics

Data was analyzed using ANOVA in GraphPad Prism followed by Tukey's test for multiple comparisons. Data is presented as mean \pm SEM. $P < 0.05$ is considered significant.

Results

Proliferation

The concentrations of 0.1 and 1.0 ng of IL-1 β had no effect on proliferation of C2C12 mouse myoblasts (Figures 1 and 2; $P > 0.05$).

Differentiation

The fusion index was decreased by 13.5% when C2C12 mouse myoblasts were treated with 1.0 ng of IL-1 β (Figures 3 and 4; $P < 0.05$). However, there was no difference in the fusion index between myoblasts treated with 0.1 ng of IL-1 β and controls (Figures 3 and 4; $P > 0.05$).

Myotube diameter

The concentrations of 0.1 or 1.0 ng of IL-1 β had no effect on myotube diameter of C2C12 mouse myoblasts (Figures 5 and 6; $P > 0.05$).

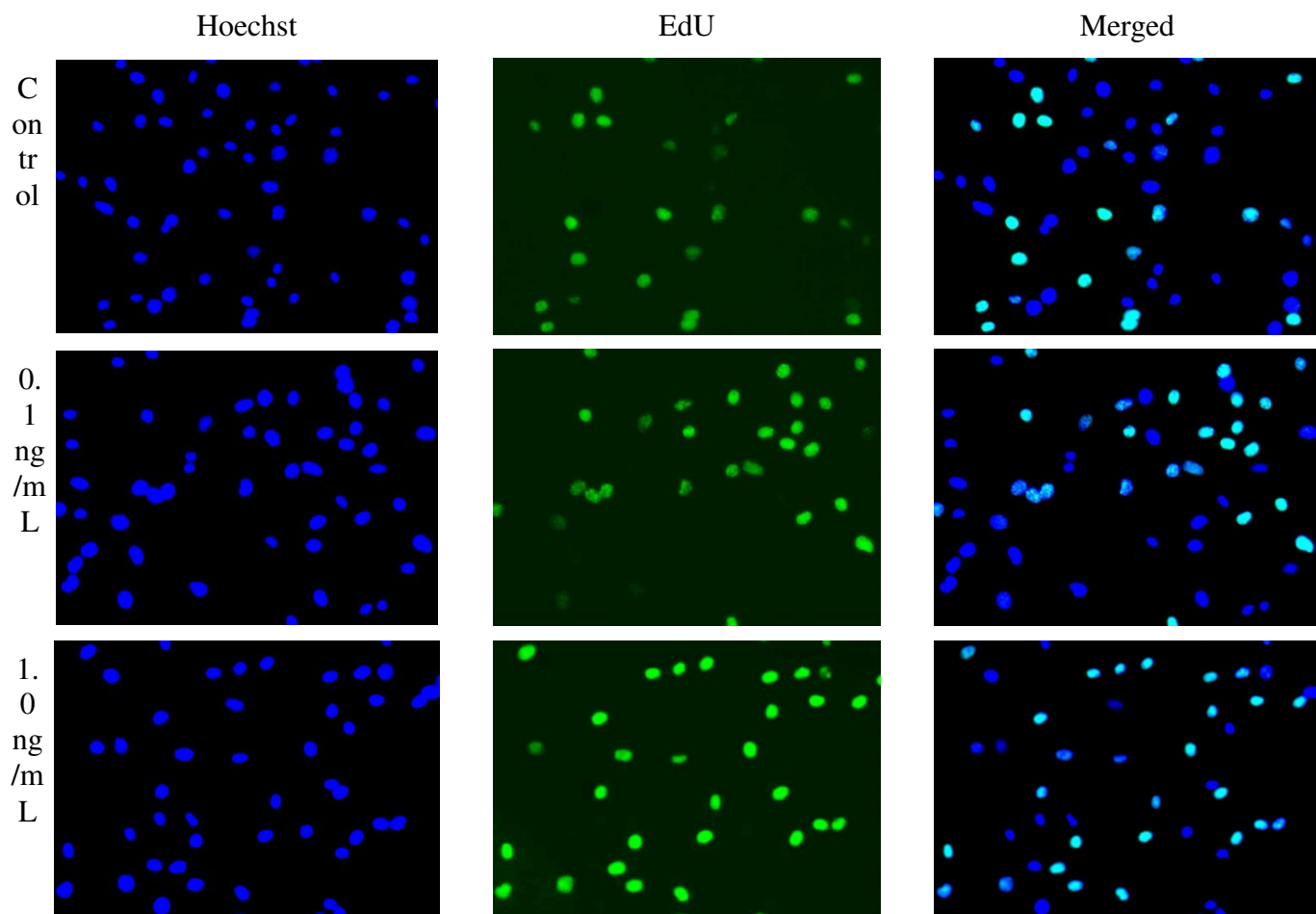


Figure 1. C2C12 mouse myoblasts proliferate in the presence of IL-1 β . C2C12s were cultured in low glucose growth media in the presence of 0.1 or 1.0 ng/mL IL-1 β or carrier only for 24 h. Cells were pulsed with edoxyuridine (green), fixed, and immunostained. Nuclei were visualized with Hoechst 33342 (blue). The two images were then merged. Representative images shown.

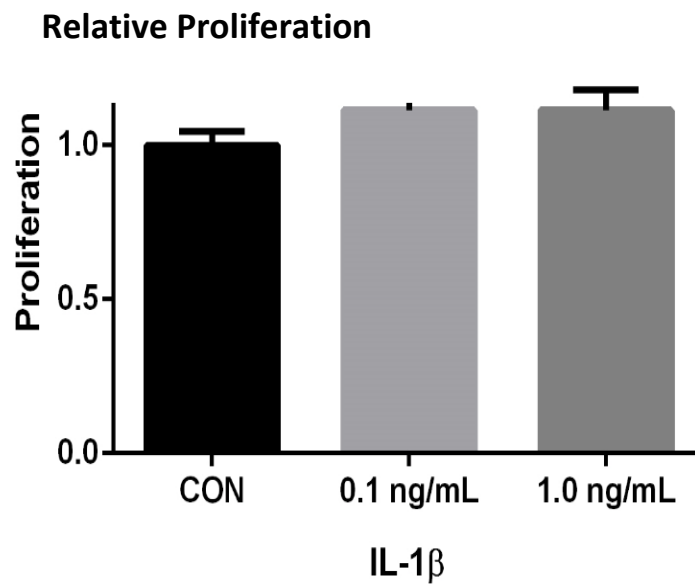


Figure 2. IL-1 β does not alter proliferation of C2C12 mouse myoblasts. The percent of total nuclei positive for EdU was calculated in C2C12s treated with 0 (CON), 0.1, or 1.0 ng/mL IL-1 β . The data is presented as means \pm SEM relative to control. n=3

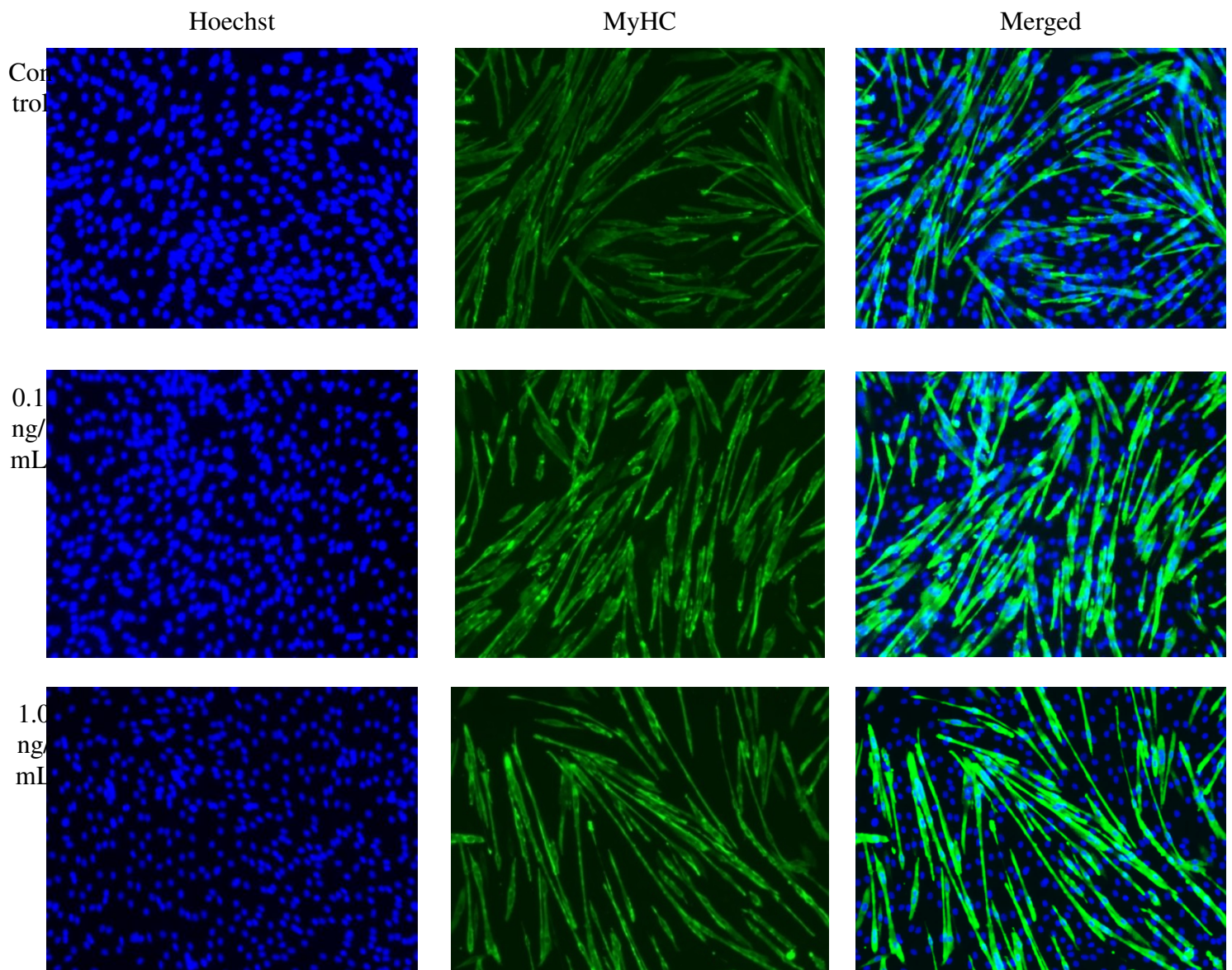


Figure 3. C2C12 mouse myoblasts differentiate in the presence of IL-1 β . Confluent C2C12 myoblasts were placed in differentiation media containing 0.1 or 1.0 ng/mL IL-1 β or carrier only for 48 h. Myotubes were immunostained for Myosin Heavy Chain [MyHC] (green) and Hoechst 33342 (blue). The two images were then merged. Representative images shown.

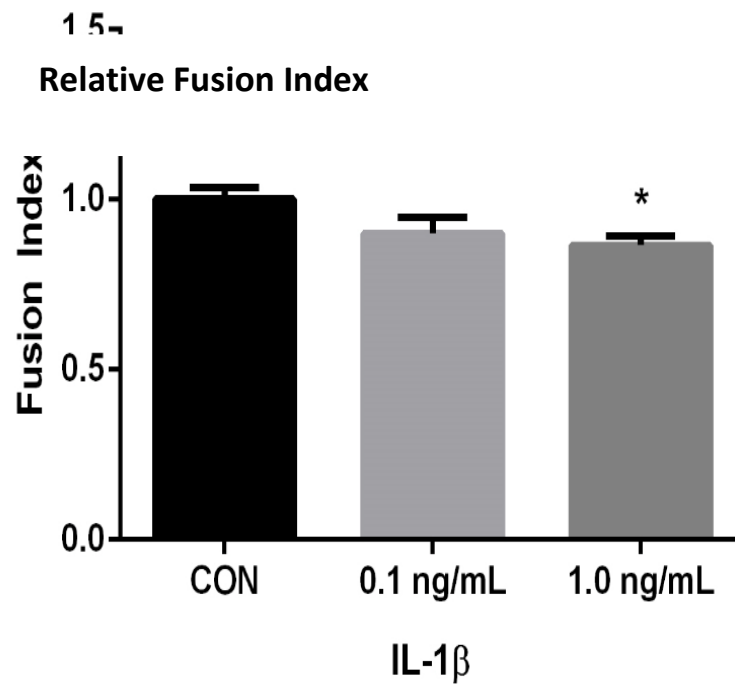


Figure 4. IL-1 β decreases fusion of C2C12 mouse myoblasts. C2C12 myoblasts were treated with 0.1 ng/mL or 1.0 ng/mL of IL-1 β or carrier only (CON). The fusion index was calculated by quantifying the number of nuclei within multi-nucleated myofibers divided by total nuclei and is presented relative to control as mean \pm SEM. * $P < 0.05$, $n=3$

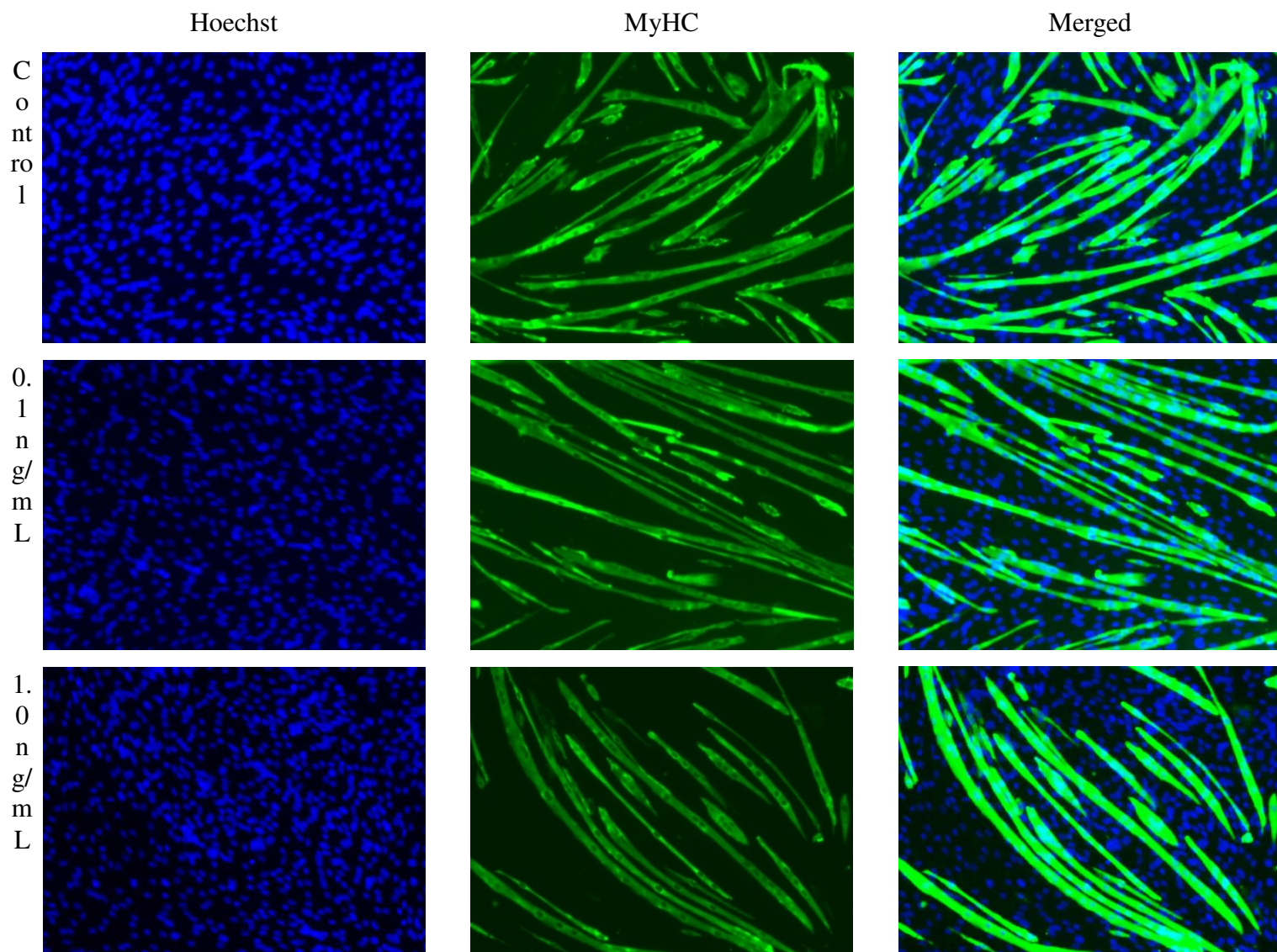


Figure 5. Myotube diameter of C2C12 mouse myoblasts in the presence of IL-1 β . Myotubes were cultured for 48 h in differentiation media and then cultured for 48 h with 0.1 or 1.0 ng/mL IL-1 β . Cells were immunostained for Myosin Heavy Chain (MyHC, green) and Hoechst 33342 (blue). The images were then merged. Representative images shown.

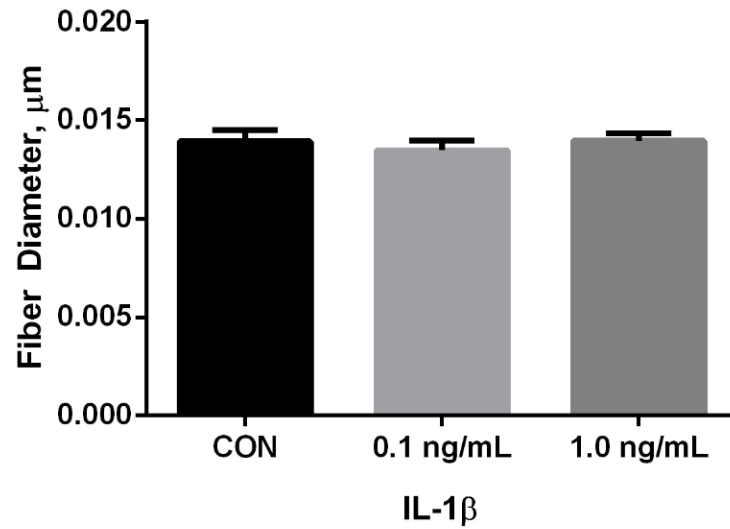


Figure 6. IL-1 β does not alter myotube diameter of C2C12 mouse myoblasts. Myotubes were cultured for 48 h in differentiation media and then cultured for 48 h with 0.1 or 1.0 ng/mL IL-1 β . Myotube diameter was measured at 3 places on each multinucleated fiber and then averaged. Data are presented as mean \pm SEM. CON=control, n=3

Discussion

The data showed that 1.0 ng/mL of IL-1 β decreases fusion index by 13.5% while proliferation and myotube diameter was not affected. Thus, IL-1 β may contribute to poor muscle growth and/or regeneration by decreasing the fusion of myoblasts into existing myofibers. It was surprising to see that IL-1 β only affected fusion index, and not myotube diameter. We hypothesized that IL-1 β would decrease fusion index and myotube diameter, since if myotubes can't differentiate properly, then most likely they would not be able to grow properly. However, IL-1 β didn't affect myotube hypertrophy, just how well they were able to fuse into fibers.

Since stress increases the production of IL-1 β (Wohleb et al., 2001), stress may contribute to poor muscle growth. Plasma concentrations of IL-1 β were increased in mice that had experienced stress, and this could potentially correlate with other animals such as livestock. Livestock experience many different types of stress such as transportation, changes in the environment or disease within their environment on a regular basis. This consistent stress could contribute to a decrease in muscle growth within the animals, leading to a decrease in muscle mass. This could lead to decreased production, which increases costs to producers and consumers.

Additionally, patients with inflammatory diseases associated with muscle weakness exhibit increases in IL-1 β (Grundtman et al., 2007). While no direct correlation was drawn between the muscle weakness and the increase in IL-1 β , the authors suggest that IL-1 β could play a major factor in the muscle weakness of these diseases. Since IL-1 β decreases differentiation, this could lead to decreased muscle growth and muscle weakness, supporting the findings of Grundtman et al. (2007). Further, Spate and Schulze (2004) showed that cancer increased IL-1 β concentrations, increased muscle atrophy, and increased inflammatory cells

within the skeletal muscle. Therefore, this muscle wasting could be due to the inflammatory cells producing more IL-1 β , inhibiting differentiation of the myofibers.

However, a study conducted by Grabiec et al. (2013) contradicted this study. When C2C12 cells were treated for three days with IL-1 β , fusion index was increased. They did treat the cells with the same concentration as this current study, but measured cyclin A and B1 as well as MyHC and myogenin which weren't measured within the current study. Therefore, the length of time treated could have caused the difference in results, but there could have been other confounding variables throughout the experiments such as the conditions the cells were grown that could give inconsistent data.

Given this information, future studies could potentially measure the pathways IL-1 β signals through. Inhibiting the JNK and p38 pathways could stop the process of signaling IL-1 β , decreasing the negative effects it has on the body. Overall, this would be able to stop the muscle wasting due to inflammation or stress resulting in stronger and healthier animals.

Conclusion

In conclusion, we found neither proliferation nor myotube diameter was affected by either concentration of IL-1 β . However, the fusion index decreased by 13.5% when C2C12 myoblasts were treated with 1.0 ng/mL IL-1 β . Thus, IL-1 β may contribute to poor muscle growth and/or regeneration by decreasing the fusion of myoblasts into existing myofibers. Since stress increases the production of IL-1 β , stress may contribute to poor muscle growth through the cascade involved with the production of IL-1 β . This can reduce the overall growth of animals in the livestock industry. Since livestock experience many different types of stress such as transportation, changes in the environment, or disease, then this chronic stress could contribute to a decrease in muscle growth within the animals, decreasing the carcass weight and meat quantity and quality.

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