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Factors Influencing Satellite Cell Activity during Skeletal Muscle Development in Avian and Mammalian Species

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ABSTRACT: Avian and mammalian skeletal muscles exhibit a remarkable ability to adjust to physiological stressors induced by growth, exercise, injury and disease. The process of muscle recovery following injury and myonuclear accretion during growth is attributed to a small population of satellite cells located beneath the basal lamina of the myofiber. Several metabolic factors contribute to the activation of satellite cells in response to stress mediated by illness, injury or aging. This review will describe the regenerative properties of satellite cells, the processes of satellite cell activation and highlight the potential role of satellite cells in skeletal muscle growth, tissue engineering and meat production. (**Key Words:** Muscle Regeneration, Muscle Development, Mitogenic Factors, Meat Production, Poultry, Mammals)

SATELLITE CELLS AS MUSCLE PRECURSORS

Throughout the process of skeletal muscle development, a large number of myoblasts combine and differentiate into muscle fibers (Charge and Rudnicki, 2004). A small population of myoblasts located between the basal lamina and sarcolemma of the emerging muscle fibers form a pool of mononuclear myogenic stem cells identified as satellite cells (Borisov et al., 2005). Muscle satellite cells emerge during the final stage of myogenesis and first appear in the limbs of an embryo (Seale et al., 2001). After the process of myogenesis is complete, the satellite cells continue to proliferate to fuel muscle growth by donating myonuclei to enlarging muscle fibers, but in mature non-growing muscle, satellite cells are mitotically quiescent (Gibson et al., 1983; Mozdziak et al., 1997; Sherwood et al., 2004). Myogenic satellite cells play an important role in posthatch and postnatal development of skeletal muscle as well as contribute to muscle regeneration. In response to myotrauma, satellite cells become activated, begin to multiply and express myogenic markers (Hawke and Garry, 2001). Several studies have demonstrated that satellite cells constitute a reserve of stem cells for regeneration of skeletal muscle. As a result of proliferative functions of satellite cells, skeletal muscle possesses exceptional regenerative properties. The satellite cells readily respond to regenerative cues such as exercise, or injury by proliferating into myoblasts, which will divide a limited number of times before terminal differentiation and fusion to form multinucleated myotubules (Wager and Conboy, 2005). New myotubes emerge only a few days following acute muscle damage. *In vivo* and *in vitro* experiments demonstrate that satellite cells are active in the regeneration of damaged muscle by fusing with pre-existing myofibers (Moss and Leblond, 1971; reviewed in Wagners and Conboy, 2005) or fusing with each other to form new myofibers (Schultz and McCormick, 1994; Bischoff, 1997; Hawke and Garry, 2001). Moreover, satellite cells can be disaggregated from single muscle fibers and grown into myotubes in a culture environment (Bischoff, 1986; Blau et al., 2001; Singh et al., 2007). The ultimate regenerative capability of satellite cells implies that they can be continuously renewed and at the same time produce differentiated progeny, suggesting that satellite cells may embody a skeletal muscle stem cell population.

SELF-RENEWAL PROPERTIES OF SATELLITE CELLS

A stem cell has the ability to self-renew and preserve at

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least one tissue throughout the life of an organism (Wagers and Weissman, 2004). Satellite cell number and regenerative power remain almost constant during multiple cycles of muscle damage and repair, implying that satellite cells self-renew (reviewed in Wagers and Conboy, 2005). The ability of existing satellite cells to provide new satellite cells was investigated by grafted freshly isolated myofibers into radiation-ablated muscles of immunocompromised, dystrophic mdx-nude mice (Collins et al., 2005). Following grafting, small and pure populations of anatomically defined satellite cells were investigated. The results have indicated that *in vivo*, a few satellite cells that were associated with one myofiber can generate a progeny of thousands of new myonuclei (Megeny et al., 1996) and they may regenerate considerably expanded numbers of functional Pax-7+ satellite cells *in vivo* (Collins, et al., 2005). Pax7 is a paired box transcription factor residing in satellite cell-derived myoblasts. Pax7 plays a very important regulatory role in development of various cell lineages (Mansouri et al., 1999). The capability of satellite cells to produce new myonuclei has been demonstrated both *in vivo* and *in vitro*. However, it is necessary to establish the ability of satellite cell self-renewal to truly define them as stem cells. The study performed by Collins et al. (2005) provides evidence that satellite cells are potent myogenic progenitors and are capable of preserving their own population by self-renewal. Satellite cells transplanted into irradiation-depleted host muscle resulted in extensive self-renewal by repopulating the muscle with myogenic cells that generated large conglomerations of newly made myofibers throughout successive cycles of injury-induced regeneration.

Additional studies of isolated single myofibers and studies of primary satellite cell isolates maintained in culture demonstrated that cultured satellite cells encompass both phenotypic characteristics of quiescent satellite cells and form clusters that contain differentiated progeny of satellite cells. Satellite cell activation and myogenic development are controlled by transcription factors including the myogenic regulatory factors Myf5, MyoD, and myogenin. Myf5 and MyoD are responsible for determination of myogenic lineage and cell activation whereas myogenin controls terminal differentiation (Zammit et al., 2004). It has been demonstrated using ex vivo explant cultures that satellite cells activated by muscle injury initiate formation of transitional progenitor cells expressing Pax7+. Subsequently, these progenitor cells divide asymmetrically and give rise to Pax3-, Myf-5+, and desmin (+) myoblasts (Conboy and Rando, 2002). Recent research has demonstrated that in cell culture systems mdx derived satellite cells engage in accelerated differentiation, indicating that some features of myogenic proliferation are self-autonomous (Yablonka-Reuveni and Anderson, 2006). Concurrently, these data suggests that satellite cells alone are able to sufficiently repair muscle.

PAX7 AS AN EARLY MARKER OF SATELLITE CELL SELF-RENEWAL AND DIFFERENTIATION

Several scientific studies performed on rodents have demonstrated that paired-box transcription factor Pax7 is expressed in satellite cells during early stages of myoblast proliferation (Seale et al., 2000; Conboy and Rando, 2002). Halevy et al. (2004) discovered the presence of the Pax7 protein in avian satellite cells suggesting that the pattern of satellite cell expression in both avian and mammalian myoblasts is autologous. In the study performed by Halevy et al. (2004), the pattern of Pax7 protein expression was analyzed in myogenic cell cultures originating from 9-day-old chickens. Double-immunofluorescence staining was employed to depict the pattern of Pax7 expression in relation to myogenin and MyoD expression to determine the presence of Pax7 protein during proliferation and differentiation of chicken myoblasts. Myogenin protein expression commences as the myoblasts begin differentiating (Zammit et al., 2004). Alternatively, MyoD protein is expressed in avian myoblasts that are in both proliferating and differentiating (Yablonka-Reuveni and Paterson, 2001). While MyoD is a marker of activated satellite cells, Pax7 expression marks mostly the proliferative reserve/quiescent population. Cell culture and *in vivo* analysis of Pax7 expression in chicken myoblasts suggests that Pax7 positive cells are mostly in pre-myogenin state. Satellite cells that are in a quiescent state may only express Pax7. In response to various stresses imposed on the muscle, the quiescent satellite cells can rapidly enter the cell cycle. During early avian muscle growth or repair, proliferating satellite cells either undergo asymmetric cell division or stochastic on/off gene switch in separate cells. In the course of asymmetric cell division, some cells undergo differentiation and some enter the reserve pool that maintains the proliferation potential of the satellite cell population. While satellite cells differentiate, myogenin expression increases and Pax7 expression gradually declines and eventually stops. Satellite cells that remain in a quiescent state may only express the Pax7 protein, which represents an early marker of myogenesis during muscle growth, and its expression is maintained in satellite cells derived from adult chickens (Halevy et al., 2004).

While satellite cells appear to be self renewing stem cells, many factors affect proliferation and regeneration of satellite cells following different types of physiological trauma, such as exercise-induced muscle injury, muscular atrophy, age-related muscular changes, muscle denervation, and disease related myopathy.

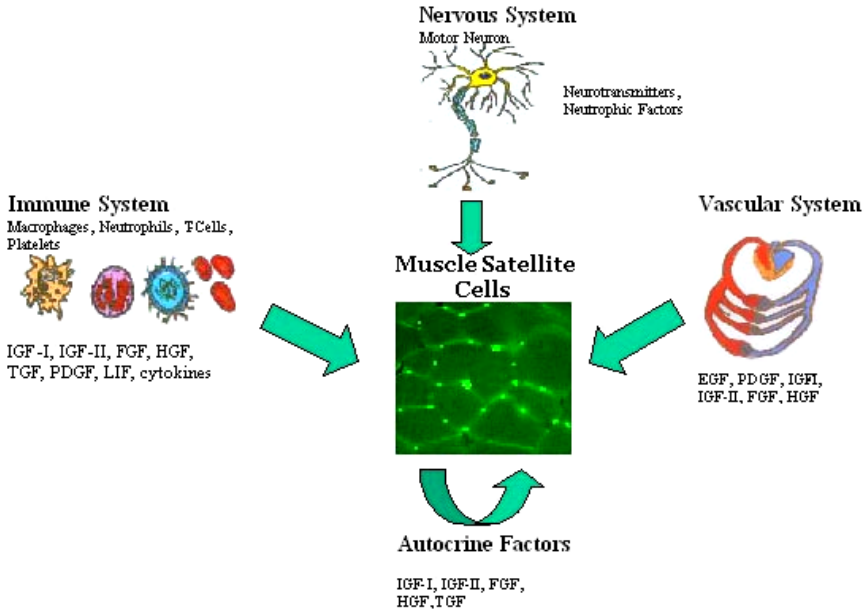

Figure 1. Factors regulating satellite cell activity. These factors regulate chemotaxis, proliferation, and differentiation of satellite cells.

REGULATION OF SATELLITE CELL POPULATION BY GROWTH FACTORS AND INFLAMMATORY PATHWAYS

Muscle regeneration requires a succession of cellular events involving various growth factors (Figure 1). The most studied growth factors that participate in satellite cell proliferation and muscle regeneration are insulin-like growth factor I (IGF-I), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), transforming growth factor (TGF) (Hawke and Garry, 2001; Nara et al., 2001), and GDF8/myostatin (Wagers and Conboy, 2005; Jin et al., 2006).

Growth factors play an important role in the regulation of proliferation, differentiation, and motility of satellite cells. Many scientific studies have utilized cell culture models analyzing the influence of growth factors on satellite cell population. The response of quiescent satellite cell activation by growth factors significantly varies depending on the age of an animal. Since satellite cell activity is higher in younger animals than mature non-growing animals, most of the satellite cell cultures are derived from early posthatch or neonatal skeletal muscle (Allen and Rankin, 1990; Zacharias and Anderson, 1991; Tatsumi et al., 1998).

The insulin-like growth factors interact with a number of cell membrane receptors including the receptors on muscle cells. Minshall et al. (1990) utilized competitive binding assays and autoradiographic analysis of hormone receptor complexes to determine the interaction of insulin-like growth factor I (IGF-I) with turkey satellite cell clones and myotubes. This study demonstrated that turkey satellite cells and satellite cell derived myotubes possess unique binding sites for insulin-like growth factor I (IGF-I) suggesting that IGF-I plays a role in satellite cell activation (Minshall et al., 1990).

Insulin-like growth factors I and II (IGF-I and IGF-II) play an important role in metabolism of insulin. These two factors are also regularly secreted by skeletal muscle and are known to increase satellite cell activation and proliferation in *in vitro* models (Allen and Boxhorn, 1989; LeRoith et al., 1992; Musaro et al., 2001). Intramuscular injection of IGF-I into older or injured animals resulted in enhancement of satellite cell proliferation and in overall increase of muscle mass (Chakravarthy et al., 2000). IGF-I is also involved in signaling pathways that participate in the regulation of the satellite cell pool through stimulation of protein synthesis. For instance, the signaling pathway that involves stimulation of satellite cells by IGF-I is mediated through phoshatidylinositol-3-OH kinase (PI-3K) (Coolican et al., 1997). It has also been determined that exercise and overload of avian and mammalian skeletal muscle elevates levels of IGF-I and at the same time an increase of DNA content implying an increase in satellite cell activity (Darr and Schultz, 1987; Carson and Alway, 1996; Adams et al., 1999).

Hepatocyte growth factor (HGF) and its c-Met receptor have also been found to have a role in the satellite cell activation. Release of HGF from damaged myofibers is proportional to the degree of muscle injury. In addition to satellite cell activation, HGF has a role in the inhibition of myoblast differentiation (Allen et al., 1995) partially by inhibition of myogenic regulatory factors such as MyoD (Miller et al., 2000).

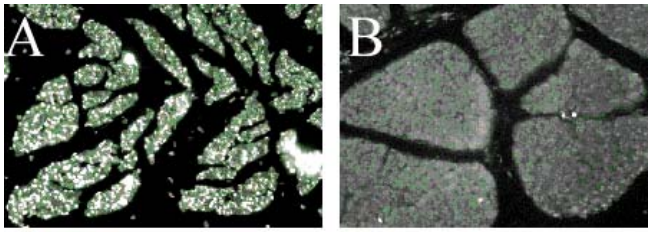

Figure 2. Pectoralis major muscle fibers stained with terminal deoxynucleotidyl tranferase in conjunction with fluorescein 12-dUTP to label apoptotic nuclei of a (A) 3-day-old feed deprived chick and a (B) 3-day-old chick provided feed.

Fibroblast growth factors consist of nine different isomers. Only FGF-6 is restricted to skeletal muscle (Sheehan and Allen, 1999). However, due to conflicting reports on the effect of FGF-6 on mouse satellite cells, the functional role of FGF-6 remains unclear. Studies have shown that hepatocyte growth factor and the FGFs-2, -4, -6, or -9, synergistically increase proliferation of satellite cells. Release of both HGF and FGF from the damaged muscle fibers is proportional to the level of injury. Additionally, the level of FGF released by the muscle and its effect on satellite cell proliferation is proportional to the expression of FGF receptor (Sheehan and Allen, 1999). McFarland et al. (2003) demonstrated that various subgroups of turkey satellite cells derived from pectoralis major muscle respond differently to mitogenic stimuli. McFarland et al. (2003) suggested that faster growing clonal satellite cell population showed higher response to FGF-2 than slow growing cell populations. In addition, turkey satellite cells with higher growth rates express elevated levels of FGF-2 and FGF receptor-1 at the beginning of the proliferation process than slower growing clones. Faster growing clones also expressed higher levels of heparin sulfate proteoglycans, which are responsible for FGF receptor signaling (McFarland et al., 2003).

Transforming growth factor-β (TGF-β) is a cytokine protein that is responsible for bone morphogenesis and growth differentiation (Allen and Boxhorn, 1989; Zentela and Massague, 1992; Kotagiri et al., 1997; Kocamis et al., 2001). The family members of TGF-β normally have a role in inhibition of muscular differentiation and proliferation by suppressing the activation of transcription of MyoD factors (Martin and Olson, 1992). It has been demonstrated that the fast growing turkey satellite cell clones are more receptive to the proliferation and differentiation depressing effects of TGF-β compared to the slow growing clones proving the inhibitory role of TGF-β on satellite cell activation during avian muscle growth (McFarland et al., 2003). However, during the process of muscle regeneration, TGF-β receptor is expressed causing an increase of cellular differentiation followed by a boost of skeletal muscle differentiation (Sakuma et al., 2000). The repair of injured muscle also involves the release of inflammatory cytokines such as interleukin -6 (IL-6), interleukin -4 (IL-4), leukemia inhibitory factor (LIF), and TNF-α (Tidball, 2005). Injured muscle induces production of macrophage and monocyte chemoattractants that creates an obstacle for inflammatory cell infiltration, which slows muscle regeneration (Bondesen et al., 2004). In LIF mutant mice, exogenous administration of LIF increased the proliferation of satellite cells and produced enlarged muscle fibers (Kurek et al., 1997). IL-6 showed no effect on activation of satellite cell population (Kami and Senba, 1998).

Growth factors play an important role in satellite cell activation in avian and mammalian muscle. However, the *in vitro* studies do not always accurately model the *in vivo* situation regarding the function of growth factors because the permissive and repressive factors regulating cell function in *in-vivo* environment are not present (Greene and Allen, 1991; Allen et al., 1995; Pavlath, 1996).

SATELLITE CELL RESPONSE TO EXERCISE-INDUCED MYOTRAUMA

Bursts of high resistance exercise cause hypertrophic growth of skeletal muscle. Muscle growth is generated by satellite cell proliferation, migratory capacity of satellite cells, and fusion to already existent myofibers. If there is a severe exercise-induced myotrauma, the basal lamina ruptures and satellite cells migrate to adjacent muscle fibers. However, if myotrauma is mild, the basal lamina remains intact and satellite cells migrate to the site of injury to begin the regenerative process (Schultz and McCormick, 1994). Exercise induced stress on the myofiber results in the spurt of blood-borne macrophages into the injured area. Macrophages orchestrate the muscle repair process by stimulating secretion of inflammatory cytokines such as IL-6 or LIF that insight satellite cell proliferation and differentiation. However, if there is no macrophage response, the repair process does not proceed (Lescaudron et al., 1999). Furthermore, exercise generated stress on muscle fibers causes an influx of IGF-I, that influences proliferation and fusion of the satellite cell pool.

SATELLITE CELL ACTIVITY IN DENERVATED AND ATROPHIC MUSCLE

Factors such as malnutrition (Figure 2), hindlimb unloading, and denervation result in atrophy of skeletal muscle that is characterized by a decrease in the number of myonuclei (Carlson and Faulkner, 1988; Kuschel et al., 1999; Dedkov et al., 2001) (Figure 2). When a normal structure of the tissue is damaged, satellite cells are able to divide and fuse resulting in the establishment of new multinucleate myofibers. Denervation of adult muscle

causes a prompt decrease in the functional capacity of the muscle followed by atrophy (Borisov et al., 2005). Previous studies have demonstrated an increase in satellite cell population shortly after denervation (Viguie et al., 1997). However, an extended period of denervation results in a considerable drop in satellite cell number (Dedkov et al., 2001). A decline in the satellite cell population may result from cell apoptosis, and limited input of factors such as IGF-I that stimulate satellite cell proliferation. Recent experiments performed by Borisov et al. (2005) suggested that progressive interstitial fibrosis and confinement of a large number of satellite cells within the endomysial tubes of atrophic muscle are the major factors preventing normal proliferation of satellite cells (Borisov et al., 2005). *In vitro* results from denervated rat extensor digitorum longus myoblasts have shown that capability of myoblasts for fusion is not irreversibly impaired subsequent to denervation. Because the release of satellite cells from their sublaminal space provides more room for active regeneration, denervated muscle is partially able to restore its functionality following injury (Borisov et al., 2005).

MUSCULAR DYSTROPHY

Muscular Dystrophy is defined as a group of over 20 genetic disorders arising from mutations in the X-linked dystrophin gene (Seale et al., 2001). Muscular dystrophies are correlated with mutations occurring in genes encoding different types of muscle proteins responsible for normal function of myofiber membrane (Imamura et al., 2000). Impairment in myofiber membrane function leads to muscle weakness and degeneration. Disease progression and death associated with muscular dystrophy are related to a failure of myogenic satellite cells to sustain muscle regeneration (Webster and Blau, 1990).

Muscular dystrophy conditions and symptoms vary across species because of differences in expression of genes responsible for this disease. Avian muscular dystrophy is characterized by a mutation in a single gene, *am* (Asmundson and Jullian, 1956). Birds with muscular dystrophy show signs of abnormalities in the growth and development of pectoralis major and other fast twitch muscles. Moreover, avian dystrophic muscle fibers exhibit variability in their diameters and are larger and more rounded than normal muscle fibers (King and Entrikin, 1991).

The chicken was an early model for human muscular dystrophy (Asmundson and Jullian, 1956). Subsequently, dystrophy was well characterized in the human and mdx mouse model (Ascadi et al., 1991; Wakeford et al., 1991), and it was demonstrated that muscular dystrophy was related to the absence of the sarcolemmal protein dystrophin in many species (Asmundson and Jullian, 1956; Gorospe et al., 1994; Iwata et al., 1996). The cytoskeleton and the extracellular matrix are joined together by dystrophin-glycoprotein complex (DGC) that is comprised of intracellular proteins such as dystrophin syntrophins, and the sarcolemmal proteins named dystroglycans, sarcoglycans, and sarcospan. When the linkage in these proteins is disrupted, sarcolemma becomes unstable and easily tears causing an increased influx of calcium into the muscle, which makes muscle fibers more susceptible to necrosis- the major pathological feature of muscular dystrophy (Cohn et al., 2002). It has been recently demonstrated that altered glycosylation and laminin-binding activity of α-dystroglycan results in muscular dystrophy in chickens (Saito et al., 2005). Mice chimeric for dystroglycan expression develop severe cases of muscular dystrophy, and it has been suggested that dystroglycan is expressed in satellite cells (Cote et al., 1999). During early phase of muscular dystrophy, skeletal muscle can efficiently repair itself. However, the continuous activation of the repair mechanism eventually drains the satellite cell proliferative capacity. As a result, inability of satellite cells to maintain muscle regeneration results in advanced fibrosis and replacement of muscle tissue with adipose tissue.

MUSCLE REGENERATION AND AGING

The self-repairing ability of injured skeletal muscle progressively decreases with age (Grounds, 1998). In avian and mammalian models, the satellite cells dynamically participate in the repair of skeletal muscle throughout the adult life, but the activity of satellite cells dramatically decreases with age (Allbrook et al., 1971; Mozdziak et al., 1994). The satellite cell population in injured skeletal muscle is also under control of evolutionary conserved Notch and Numb signaling pathways, which play a key role in age-related muscle regeneration (Conboy and Rando, 2002). These pathways play an important role in embryonic organogenesis. While proliferation of satellite cells in response to muscle injury is upregulated by the Notch pathway, the terminal myogenic differentiation is restrained by the Numb pathway, which functions as a metabolic antagonist of Notch. During muscle injury, Notch ligand, Delta, binds to its receptor and activates Notch signal transduction, which simultaneously stimulates mitotic activity of satellite cells (Conboy and Rando, 2002). As age progresses, Notch ligand, Delta, fails to sufficiently transduce signals to the Notch receptor, resulting in incompetent regeneration of old muscle tissue (Conboy et al., 2003). Results of studies performed on old and young rodents have suggested that age-related decline in regenerative capabilities of satellite cells are dependent on the cell environment. Muscle transplantation studies between young and old animals have demonstrated that the

recovery of a muscle is dependent on the age of the host rather than on the age of the transplanted muscle (Carlson and Faulkner, 1989). It was observed that Notch signaling pathway and subsequent activation of satellite cells have been restored in the old tissue due to exposure to the internal environment of a young organism (Conboy et al., 2005). The reconstitution of the proliferative capacity of aged satellite cells by exposure to the young systemic environment may be crucial for future therapies focused on restoration of regenerative properties of stem cells in aged population.

TISSUE ENGINEERING AND ENHANCING MEAT PRODUCTION

Tissue engineering holds promise in the restoration of lost, damaged or failing tissues or organs. While several tissue engineering techniques attempt the rebuilding of human organs have been used in medical practice, the reconstruction of skeletal muscle is still under investigation. Since satellite cells can be harvested from adult muscle and grown *in vitro*, skeletal muscle tissue engineering relies on regenerative properties of these cells and their proliferative potential. Normally, satellite cells are in a quiescent and undifferentiated state. When stimulated, these progenitor cells enter mitosis, which induces division and fusion of myoblasts to form myotubes that consequently assemble into muscle fibers (Reviewed in Bach et al., 2004). Primary satellite cell cultures may better reflect muscle development than myogenic cell lines (Marzaro et al., 2002). Successful transplant of tissue created *in vitro* into an *in vivo* environment requires that the donor cells be autologous, or as a minimum, non-immunogenic. Hence, it has been suggested that autologous primary satellite cells are ideal candidates for muscle tissue engineering (Bonassar and Vacanti, 1998). The reconstruction of skeletal muscle tissue utilizing tissue-engineering techniques has a great potential in the treatment of skeletal muscle diseases such as muscular dystrophy and spinal muscular atrophy, severe muscle injuries, tumor ablations, and prolonged muscle denervations (Bach et al., 2004) because it may be possible to expand the autologous cell populations derived from healthy muscle *in vitro* and introduce them into injured areas.

Studies focusing on muscle development in poultry have demonstrated an age-related decrease in satellite cell mitotic activity (Mozdziak et al., 1994) because satellite cell mitotic activity was high during the early phases of growth, and it fell to low levels at approximately 9 weeks of age. Therefore, manipulation of satellite cell mitotic activity early in life may have a great impact on muscle development in meat producing animals. Several studies have revealed that satellite cell mitotic activity can be suppressed or stimulated by various physical and nutritional factors (Mozdziak et al., 1997; 2000; Dangott et al., 2000; Pophal et al., 2004). Inhibition of satellite cell activity by irradiation and hindlimb unloading in young animals results in smaller myofiber diameter and lower muscle size at maturity (Mozdziak et al., 1997; 2000). Nutritional stimulation of satellite cell mitotic activity may also result in higher muscle yield. It has been recently shown that the level of satellite cell mitotic activity in early post-hatch chickens can be influenced by treatment with different amounts of dietary lysine (Pophal et al., 2004). An earlier study by Dangott et al. (2000) has demonstrated that dietary creatine supplementation in combination with an increased functional load stimulated satellite cell mitotic activity in rats, and that the nutritional supplementation was correlated with a larger myofiber diameter compared to non-supplemented control animals. Taken together, the results of these studies suggest that it is possible to influence muscle size by nutritionally or functionally targeting the satellite cell population.

Further studies focusing on the molecular pathways regulating satellite cell self-renewal and differentiation are crucial for developing therapies for the improvement of the regenerative capabilities of the skeletal muscle lost due to disease or aging. Since most of the research analyzing factors responsible for satellite cell activation was performed in the in-vitro environment, future challenges include implementation of the in-vitro processes into an *in vivo* situation. In depth understanding of the mechanistic processes governing satellite cell activity could also be advantageous in muscle growth augmentation in domestic animals. Because satellite cell activity peaks during early stages of muscle development, manipulating satellite cell population and stimulation of biochemical factors influencing satellite cell proliferation by dietary treatments immediately post-hatch may improve muscle growth and quality later in life. Consequently, understanding the influence of satellite cells on muscle growth could lead to more optimal meat production at market age.

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