Review

The Role of Prostaglandin $F_{2\alpha}$ in Skeletal Muscle Regeneration

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Prostaglandins are a diverse group of eicosanoid hormones that modulate various processes, many of which are related to inflammation. Due to this, prostaglandins are the target of cyclooxygenase (COX) inhibitor drugs. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) mediates processes integral to successful skeletal muscle regeneration, roles elucidated through suppression of its production with various COX-inhibitors.

Objective: To provide a comprehensive review of literature regarding roles for the COX-metabolite $PGF_{2\alpha}$ in skeletal muscle regeneration and consequences of its suppression with COX-inhibitors.

Design: Systematic review.

Method: Recognition was given to early in vitro studies that first established roles for $PGF_{2\alpha}$ in two specific regenerative processes, and attention was then directed to human experiments investigating the $PGF_{2\alpha}$ response to aerobic and resistance exercise.

Results: $PGF_{2\alpha}$ mediates protein synthesis and satellite cell activity post-injury. Although methodological differences exist between experiments, research has unanimously demonstrated COX-mediated suppression of $PGF_{2\alpha}$ diminishes these regenerative processes.

Conclusions: All experiments in humans have been acute exercise interventions. Studies involving repeated exercise and repeated administration of COX-inhibitors seem warranted to determine if chronic use impedes skeletal muscle regeneration after exercise. Such a finding may hold serious implications for recreational athletes, patients, and clinicians managing musculoskeletal pain or diseases with regular use of COX-inhibitors.

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Key words: exercise-induced inflammation ■ eicosanoid hormones ■ analgesics ■ non-steroidal anti-inflammatory drugs (NSAIDs)

When hormones that mediate the development of skeletal muscle strength and hypertrophy are considered, anabolic steroids, growth hormone, and insulin-like growth factor-1 typically receive much attention. However, as the study of exercise endocrinology has expanded, roles for lesser known chemical messengers in anabolic processes have been discovered. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is one such hormone, described as an endogenously-produced growth factor.[1, 2] $PGF_{2\alpha}$ is a member of the prostaglandin family of eicosanoid hormones. Prostaglandins have dual roles in regeneration and inflammation, but it is their activities in inflammation that make them the primary target of cyclooxygenase (COX)-inhibitor drugs (Table 1). Indeed, much of the research that seeks to elucidate the roles of prostaglandins in skeletal muscle regeneration does so through suppression of its production with COX inhibitors.

Prostaglandins have pleiotropic effects in a range of cell and tissue types.[1] Prostaglandins sensitize nociceptors in the pain response (PGE_2)[3] and contribute to vasodilation and control of local blood flow control during exercise (PGE_2 and PGI2).[4-6] In regards to roles in regeneration, the COX-pathway and its metabolites, specifically prostaglandins E and F, have been shown to stimulate in vitro and in vivo satellite cell proliferation, differentiation, fusion, myonuclear accretion, and mixed muscle protein synthesis in both human and animal models of injury.[1, 2, 7-17]

The purpose of this article is to present literature describing roles for the COX-metabolite $PGF_{2\alpha}$ in regenerative processes in skeletal muscle. With $PGF_{2\alpha}$ activities inferred through consequences of its suppression with COX-inhibitors, some discussion of these drugs will also be provided, as they themselves have been the focus of much research. Due to the fact prostaglandins are induced by inflammation, various techniques have been employed throughout the body of research to elicit inflammation. Pathophysiological differences between contraction-induced injury and those resulting from strains, bruises, and lacerations must be acknowledged, as it is possible these differences account for contradictory findings between some human and animal research. Studies using non-exercise models of injury are included in this manuscript in light of the fact that COX-inhibitors are often used to ameliorate pain associated musculoskeletal injuries aside from exercise. Considering the relative ease with which COX-inhibiting drugs can be procured and their use for maladies ranging from delayed-onset muscle soreness to arthritis and headaches, awareness of the potential consequences of prostaglandin suppression are important to many individuals, including athletes,

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recreational exercisers, and the elderly. While pharmaceutical advancements have allowed for selective inhibition of either or both COX- isoforms, prostaglandins inhibition is non-selective, indicating production of all prostaglandins is inhibited. While suppression of those whose prominent role lies in potentiation of inflammation and pain are certainly useful, suppression of others with roles in regeneration may be questionable.

Prostaglandin Synthesis

Mechanical deformation of the skeletal muscle membrane leads to an increase in intracellular calcium with consequent activation of phospholipase A_2, which cleaves the prostaglandin hormone substrate, arachidonic acid, from the membrane. Once free, arachidonic acid may be converted into leukotrienes through the lipoxygenase pathway or prostaglandins or thromboxanes through the COX pathway. Exactly what controls entry into a specific pathway remains speculative.[18]

Within the COX pathway, arachidonic acid is acted upon by the COX enzyme, producing cyclic endoperoxide intermediates - PGG_2 and PGH_2. PGH_2 is then acted on by various isomerases to produce the various prostaglandin subclasses (Figure 1). Exactly which prostaglandin is produced depends on enzymes present in the biosynthetic pathways.[3] Prostaglandin synthesis can be regulated by controlling the activity of phospholipase A_2 or the COX enzyme. These two different control mechanisms account for the differing actions of corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) (COX-inhibitors), with steroids acting early to inhibit phospholipase A_2 through production of lipocortin[3], and NSAIDs acting later to inhibit the COX enzyme, thereby inhibiting prostaglandin production. Corticosteroids inhibit the formation of all metabolites of lipoxygenase and cyclooxygenase pathways,[19] while NSAIDs inhibit formation of COX-metabolites only.[3]

Prostaglandins are generally believed to operate in autocrine/paracrine fashion; however, prostaglandins A, E, and I may reach the systemic circulation and exert effects at distance sites.[3, 20] Intensity of mechanical deformation has been shown to affect prostaglandin release,[4] and concentrations as low as 1μg/L are capable of producing physiological actions.[18] While it has been suggested virtually all mammalian cells, except erythrocytes, may produce and respond to prostaglandins,[3] they are not considered to exist pre-formed in any cellular reservoir.[18, 21, 22]

Prostaglandins mediate signaling through seven-membrane spanning G protein-coupled receptors which are distinct for each prostaglandin. In vitro examination of mouse myoblasts has demonstrated skeletal muscle cell growth is mediated through the $PGF_{2\alpha}$ receptor, FP.[1] Formation of the hormone-receptor complex ($PGF_{2\alpha}$ – FP) results in increases in second messengers calcium, inositol 1,4,5-triphosphate (IP_3), and diacylglyceride (DAG),[3] with the cytoplasmic increases in calcium linked to activation of the transcription factor nuclear factor of activated T cells (NFAT).[1]

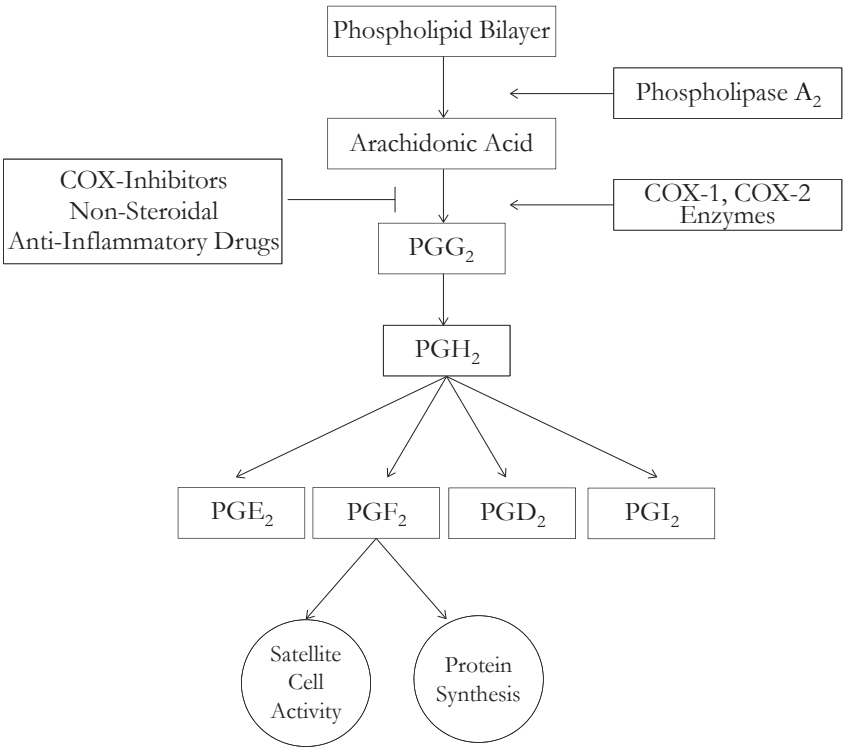

Figure 1 Mechanism of Prostaglandin Synthesis and COX-Inhibition

Table 1 Non-Steroidal Anti-Inflammatory Drug COX Classification System

Non-Selective Chemical Name (*Brand®*)	COX-2 Selective Chemical Name (*Brand®*)	COX-1 Selective Chemical Name
Diclofenac (*Voltaren*)	Celecoxib (*Celebrex*)	Aspirin
Diflunisal (*Dolobid*)	Valdecoxib (*Bextra*)	SC-560
Etodolac (*Lodine*)	Rofecoxib (*Vioxx*)	FR122047
Fenoprofen (*Nalfon*)	NS-398	Mofezolac
Flurbiprofen (*Ansaid*)		P6
Ibuprofen (*Motrin, Advil*)		TFAP
Indomethacin (*Indocin*)		
Ketoprofen (*Orudis*)		
Ketorolac (*Toradol*)		
Naproxen (*Aleve, Naprosyn, Anaprox, Naprelan*)		
Oxaprozin (*Daypro*)		
Piroxicam (*Feldene*)		

Cyclooxygenase Enzyme

The COX enzyme exists in two isoforms (COX-1 and COX-2), and COX-inhibitors are classified as COX-1 selective, COX-2 selective, or non-selective, indicating inhibition of both isoforms (Table 1). COX-1 is often described as a constitutive enzyme. It is present in many body tissues, and its concentration remains largely stable, even in response to injury.[23] In contrast, COX-2 is low or absent in resting cells[13, 19] but is significantly increased in response to various form of muscle injury and subsequent inflammation.[3, 7, 8, 13] Satellite cells have been shown to express COX-2 after injury.[8, 13] While literature presented herein presents research investigating a COX metabolite, COX enzymes themselves have been the focus of much research, with the results of inhibition of one or both isoform being conflicting across human and animal studies employing different forms of injury.[23, 12, 24, 13, 10, 8]

A selective COX-2 inhibitor (celecoxib) administered in 3 doses (200 mg/dose) over a 24-hour period was not found to affect mixed muscle protein synthesis in recreationally-active males after a single bout of resistance exercise;[23] however, a non-selective COX-inhibitor (indomethacin) administered in three doses (400 mg/dose) over a 24-hour period was reported to significantly inhibit skeletal muscle fractional synthesis rate in recreationally-active males after a single bout of resistance exercise.[15] A selective COX-2 inhibitor (celecoxib) was also not found to decrease satellite cell number when administered twice a day (200 mg/dose) for nine days after a single bout of resistance exercise performed by recreationally-active males and females.[24] Thus, while some human research seems to support a predominant role for COX-1 in human protein synthesis and satellite cell activity, animal studies support a predominant role for the COX-2 isoform in these processes.

COX-2 gene deficient mice (male, 8-11 week old) are reported to exhibit myoblasts 31% smaller than normal mice, while myoblasts size is normal in COX-1 gene deficient mice.[13] Prophylactic treatment with a selective COX-1 inhibitor (SC-560, 3 mg/kg/day, beginning 3 days pre-freeze injury) did not affect myoblast proliferation; however, a similar treatment regimen with a selective COX-2 inhibitor (SC-236, 6 mg/kg/day) did significantly decrease proliferation.[13] Another selective COX-2 inhibitor (NS-398, 5 mg/kg/day or 10 mg/kg/day for 3 or 5 continuous days) given post-laceration injury has also been shown to inhibit satellite cell proliferation and differentiation in mice (4 weeks old).[10] Animal research employing a contraction-induced form of injury (synergist ablation) reported a 75% reduction in hypertrophy and 20% reduction in satellite cell proliferation in male mice (10-15 weeks old) treated with a selective COX-2 inhibitor (NS-398, 10 mg/kg/day) daily for 14 days.[8] An in vitro investigation of 9-month old rat hind limb muscles cultured with three different COX-inhibitors for 96 hours (NS-398, SC-560, and naproxen sodium at a 1:1000 solution concentration) found selective inhibition of COX-2 (NS-398) and non-selective inhibition of both COX-1 and COX-2 (naproxen sodium) significantly decreased proliferation, differentiation, and fusion.[9] Selective inhibition of COX-1 (SC-560) also resulted in significant decreases in differentiation and fusion but was not found to adversely affect proliferation.[9] In vitro research using both pharmacological (SC-236) and genetic approaches (COX-2 gene deficient) to manipulate COX-2 substantiated the importance of COX-2 in satellite cell activity, with both techniques resulting in decreased myoblast proliferation after 5

hours of cyclic stretch stimulus.[7] Thus, in contrast to human research, animal research seems to support the COX-2 pathway in various stages of the satellite cell cycle and regeneration.

Prostaglandins and Mixed Skeletal Muscle Protein Synthesis

One of the proposed primary effects of mechanical activity on protein synthesis is the release of arachidonic acid from the skeletal muscle phospholipid bilayer, with subsequent changes in protein synthesis due to the influence of arachidonic acid metabolites (COX metabolites) formed within the COX biosynthetic pathway $PGF_{2\alpha}$ and PGE_2 have been shown to mediate protein synthesis and protein degradation, respectively.[16, 17] While unstimulated in vitro tissue incubated in the absence of arachidonic acid demonstrated no changes in protein metabolism,[17] application of exogenous arachidonic acid resulted in increases in protein synthesis, similar to those seen in stimulated tissue.[16] However, it is not arachidonic acid itself, but rather its metabolites, that stimulate protein metabolism, as 11,14,17-eicosatreinoic acid (a compound that is a structural analog to arachidonic acid but unable to serve as a COX enzyme substrate) was found to have no effect on protein synthesis or degradation.[17] Human and animal research has demonstrated COX metabolites PGE_2 and $PGF_{2\alpha}$ increase locally as a result of mechanical deformation,[4, 6, 7, 14-16, 25-27] and protein synthesis and degradation are then modulated through metabolites of this membrane constituent.[7, 14-17]

Some of the initial research illuminating prostaglandins' role in protein metabolism was a multi-faceted study employing both in vitro and in vivo examinations of isolated rat muscles (skeletal, atrial, and diaphragm).[17] In vitro experiments revealed exogenous arachidonic acid significantly increased protein degradation (nmol/mg muscle/2 hours) 20-42% in the diaphragm, soleus, EDL, and atrial muscles, while only the soleus exhibited significant increases in protein synthesis. Indomethacin (10^{-5}, 2 x 10^{-7}, 2.8 x 10^{-6} M), aspirin (5 x 10^{-4} M) , meclofenamate (10^{-5} M) administered to these in vitro tissues abolished the metabolic effects arachidonic acid, reducing protein degradation and synthesis.[17] The soleus response to arachidonic acid could allude to a possible difference in prostaglandin synthesis between fast and slow twitch muscle fibers.[16] Although there is no consensus linking fiber type and prostaglandin response,[28] fast twitch muscle fibers have greater potential for hypertrophy than slow twitch due to a greater density of androgen receptors.[29]

In an attempt to determine which specific arachidonic acid metabolites mediate protein synthesis and degradation, production of six known metabolites was measured using radioimmunoassay.[17] Only PGE_2 and $PGF_{2\alpha}$ were found to have significant effects on protein metabolism, with PGE_2 consistently stimulating protein degradation 22% and $PGF_{2\alpha}$ consistently increasing protein synthesis 35%. This finding was extended to in vivo models, in which arachidonic acid stimulated PGE_2 and $PGF_{2\alpha}$ production 5-6 fold and 3-fold, respectively[17] Consistent with in vitro data from the same experiment, in vivo levels were dramatically reduced upon addition of indomethacin (2.8 x 10^{-6} M),[17] which coincidentally, has been shown to exert greater inhibitory effects on $PGF_{2\alpha}$ than PGE_2.[30]

In vitro examination of rabbit forelimb muscles subjected to either intermittent mechanical stretching or constant tension for 90 minutes further substantiated the role $PGF_{2\alpha}$ in protein synthesis.[16] Intermittent mechanical stretching significantly increased rates of protein synthesis (%/day) compared to muscles held under constant tension, while exogenous $PGF_{2\alpha}$ added to muscles held under constant tension also resulted in significant increases in protein synthesis. Indomethacin (50 μM) and meclofenamic acid (50 μM) reduced protein synthesis 42.2% and 35.4%, respectively in the intermittently stretched muscle.[16] Activation of muscle protein synthesis has been reported to persist 10-20 minutes after removal of a 30 minute stretch stimulus, in what has been termed residual activation, with the explanation that excess arachidonic acid released upon stimulation is retained intracellularly and continues to be metabolized into various prostaglandins.[16] This acute activation has been reported to decline progressively after stimulus removal, with the temporal sequence likely differing between models of mechanical stimulation.[28]

Human research has demonstrated a single bout of resistance exercise performed by recreationally-active fasted male and females (n=8) results in elevations in mixed muscle protein synthesis of 112%, 65%, 34% at 3-, 24-, and 48-hours.[31] The coincident catabolic process of mixed muscle protein degradation was also significantly elevated 3- and 24-hours post-acute resistance exercise but was reduced to baseline by 48 hours.[31] In comparison to the 65% increase reported in males and females 24 hours after a single bout of resistance exercise, slightly higher rates of mixed muscle protein synthesis have been reported in recreationally-active males 24 hours post-acute resistance exercise.[15] While mixed muscle protein synthesis was elevated 76 ± 19% in placebo-treated males, protein synthesis was unchanged in males treated with maximal non-prescription doses of ibuprofen (3-400 mg/doses) and acetaminophen (2-1,500mg/doses plus 1-1000mg/dose) orally administered over a 12 hour period.[15] To understand the mechanism by which mixed muscle protein synthesis was attenuated by these COX-inhibitors, the same biopsies used for measurement of protein synthesis were analyzed for $PGF_{2\alpha}$ concentrations. Again, placebo-treated males exhibited 77% increases in $PGF_{2\alpha}$ biopsy concentrations in response to a single bout of resistance exercise, but levels were unchanged in males treated with both ibuprofen and acetaminophen,[14] providing further support to demonstrate $PGF_{2\alpha}$ stimulates mixed muscle protein synthesis.[16, 17]

Participant training and nutritional status are important to consider when interpreting literature on basal and exercise-induced rates of mixed skeletal muscle protein synthesis. Cross-sectional comparison of trained and untrained males and females reported no difference in fasted-state basal mixed muscle protein synthesis between trained and untrained participants;[32] however, longitudinal examination of the effects of repeated resistance exercise on fasted-state basal mixed

muscle protein synthesis in initially untrained males found 8-weeks of unilateral resistance exercise increased metabolism in trained musculature.[33] While basal mixed muscle protein synthesis was increased ~48% as a result of training, basal myofibrillar protein synthesis was similar between untrained and trained muscle. The exercise-induced responses in mixed muscle protein synthesis are similar between cross-sectional and longitudinal data, with trained participants exhibiting comparatively smaller increases in mixed muscle protein synthesis than untrained participants and an attenuated response after 8-weeks of progressive resistance training.[32, 33] Acute resistance exercise has been shown stimulate myofibrillar protein synthesis to the same degree in trained and untrained muscle.[33] Additionally, the nutritional status of participants influence measurements of protein synthesis and are important to consider, as the effects of feeding are synergistic to the effect of exercise on protein metabolism. The studies pertaining to protein metabolism within this review were conducted with participants in a fasted state.

With numerous studies demonstrating the connection between $PGF_{2\alpha}$ and protein synthesis, the possibility of attenuating the anabolic response to exercise through repeated $PGF_{2\alpha}$ inhibition was suggested.[14] To address this, venous blood samples were obtained from recreationally-trained males before and after repeated bouts of resistance exercise performed twice weekly for six weeks and coupled with prophylactic (30 minutes prior to exercise) treatment with placebo or naproxen sodium (440mg). Resistance exercise resulted in an acute increase in $PGF_{2\alpha}$ metabolites in placebo-treated males, while this response was significantly reduced by naproxen sodium (unpublished data). While this acute $PGF_{2\alpha}$ reduction due to a non-selective COX-inhibitor confirms other research, the acute $PGF_{2\alpha}$ response to resistance exercise diminished over time, and gains in strength (1RM testing), skeletal muscle tissue (DXA), and dominant arm hypertrophy (Gulick® tape measure) were not different between placebo- and naproxen-sodium treated participants (unpublished findings). This lack of effect on performance and anthropometric parameters supports other research in which a single dose of ibuprofen (400mg) given immediately after unsupervised unilateral resistance training performed by experienced male and female participants five days per week for six weeks did not affect muscle thickness (ultrasound) or strength development (1RM).[34]

Prostaglandins and Satellite Cell Activity

Satellite cells are a population of muscle-derived stem cells whose activity provides potential for skeletal muscle repair after injury.[9, 35] Satellite cells provide new myonuclei during hypertrophy and assist in repair of damaged muscle fiber segments.[9, 11] Once activated, satellite cells give rise to myoblasts, which sequentially proliferate, differentiate, align, and fuse to allow regeneration. Satellite cells examination typically involves chemiluminescent staining for various markers associated with different stages of the cell cycle, including BrdU, CD56/NCAM, FA1, Ki-67, myogenin, MHCn, desmin, and Pax7.[1, 7, 8, 10, 11, 26, 35-38] There is still debate concerning the ideal satellite cell marker,[39] and training status may influence which marker is more appropriate, as FA1 has been shown to stain 25% more satellite cells in untrained than trained individuals.[12]

Some of the seminal research highlighting the role of PGs in human myogenesis was an in vitro study of myoblasts from fetal leg muscles.[2] Exogenous $PGF_{2\alpha}$ stimulated myoblast proliferation, which was most evident 24-48 hours post-$PGF_{2\alpha}$ addition. Indomethacin and aspirin (10-20μL in aqueous solution) administered to myoblasts resulted in initial inhibition of proliferation as early as 24 hours, with complete inhibition 48-72 hours after exposure.[2] Surprisingly, smaller daily doses of indomethacin (2×10^{-5} M) were found to have greater inhibitory effects of proliferation than a larger single large dose.[2] Indomethacin and aspirin also significantly reduced the rate of fusion, even when added to culture media one day after the initial onset of fusion; however, maximal inhibitions of fusion were achieved when cells were exposed to COX-inhibitors 24 hours before the expected onset of fusion.[2]

An in vitro study of differentiating adult mice tibialis anterior muscles cultured and treated with placebo or $PGF_{2\alpha}$ demonstrated that not only did $PGF_{2\alpha}$-treated myoblasts appear larger at 48 hours, they also demonstrated a significant increase in the proportion of myotubes expressing five or more nuclei per fiber 48 hours after administration of exogenous $PGF_{2\alpha}$.[1] This study reported no effect of $PGF_{2\alpha}$ on proliferation or differentiation but significant increases in fusion due to enhanced myonuclear accretion, with $PGF_{2\alpha}$ potentially affecting any phase(s) of fusion, including motility, alignment, recognition, adhesion, or membrane union.[1] Human research has substantiated the importance of myonuclear accretion in the hypertrophic process that occurs during adulthood.[38] The effects of $PGF_{2\alpha}$ have been postulated to be dependent on the developmental stage of the myotube, enhancing fusion during embryonic development and protein accretion during regeneration from injury.[1] In regards the contradictory results pertaining to the role of $PGF_{2\alpha}$ on satellite cell proliferation, differentiation and fusion, differences in cell type (human fetal leg myoblasts (80-94 days old) versus differentiating adult mice tibialis anterior muscles) likely account for the discrepancies between the studies.[1,2]

Acute resistance exercise performed by sedentary males (n=8) has been shown to result in significant increases in $NCAM^+$ and $FA1^+$ cells 4 and 8 days post-exercise,[35] as well as $Pax7^+$ cells 8 days post-isokinetic resistance exercise in recreationally-active males (n=8).[11] Acute aerobic exercise performed by trained males has been shown to result in a significant increase in $NCAM^+$ cells 8 days post 36km run.[12] Administration of a non-selective COX-inhibitor (indomethacin) has been shown to significantly attenuate the number of cells expressing satellite cell markers in humans after acute resistance and aerobic exercise.[11, 12] Indomethacin (100mg day) treatment beginning 4 days prior to a 36-km run and continuing for 9 days resulted in lower $NCAM^+$ cells one and three days post-36K, but differences between placebo- and indomethacin-treated were not statistically different until day

8, at which point satellite cells were 27% lower in those treated with indomethacin.[12] Significant reductions in satellite cell number after acute resistance exercise was also reported in another study in which indomethacin was infused directly into active musculature (45 mg over 7.5 hours).[11]

Significant reductions in satellite cell activity upon administration of selective COX-2 inhibitors (NS-398, SC-236) have also been reported in animal experiments.[8, 10, 13] Muscles from 4-week old mice were grown in proliferation and differentiation medium along with different concentrations of NS-398 (1, 10, or 100 μmol/L).[10] In vitro proliferation was significantly reduced on days 3 and 4 by 100 μmol/L NS-398, while differentiation was significantly reduced on day 2 by both 10 and 100 μmol/L NS-398 concentrations. All concentrations of NS-398 significantly inhibited supernatant-derived $PGF_{2\alpha}$ concentrations collected from differentiation experiments.[10] In vivo data from the same study entailed laceration of the gastrocnemius along with intraperitoneal injection of 5 mg/kg or 10 mg/kg bodyweight for 3 or 5 continuous days after laceration. Microscopic examination of muscles 7- and 14-days post-laceration revealed not only were more regenerating fibers present in non-treated controls but their nuclei were significantly larger than all NS-398 treated groups (5 mg/kg for 3 and 5 days as well as 10 mg day for 3 days).[10] NS-398 treatment (10mg/kg/day for 14 days) has also been reported to significantly reduce $BrdU^+$ positive cells 5 days after synergist ablation in 10-15 week old male mice.[8]

Adult satellite cells obtained from isolated rat skeletal muscle were grown in the presence of naproxen sodium (non-selective COX-inhibitor), NS-398 (selective COX-2 inhibitor), or SC-560 (selective COX-1 inhibitor) 24 - 120 hours.[8] Proliferation was significantly increased at 72-120 hours in control cells; however, COX-inhibitors were found to significantly decrease satellite cell activity. Inhibition of COX-2 alone (NS-398 treatment) decreased proliferation, while inhibition of both COX isoforms (naproxen sodium treatment) decreased both differentiation and fusion. These findings support other research to demonstrate a 20% reduction in proliferating myogenic cells in NS-398 treated mice[8] but contradict another to report no effect of a non-selective COX-inhibitor (naproxen sodium) on satellite cell proliferation in female rat skeletal muscle subjected to contusion injury.[37] A difference in experimental design (in vivo contusion versus in vitro satellite cell activity) may explain the difference in the results.

In vitro examination of cultured adult male mice myoblasts (8-11 weeks old) subjected to 5 hours of cyclical stretch provided additional evidence of the importance of COX-metabolites in satellite cell activity.[7] In response to stretch, proliferation increased 32% by 24 hours and continued to increase to 41% by 48-hours, which is similar to animal research that has reported a residual elevation of protein synthesis up to 48 hours after in vivo eccentric work and immobilization models of injury.[40, 41] Supernatants collected from stretched myoblasts and administered to non-stretched myoblasts resulted in a 27% increase in proliferation; however, addition of SC-236 (COX-2 selective) resulted in failure to proliferate upon stretch stimulation.[7] In fact, in cells treated with SC-236, proliferation actually decreased compared to non-stretch controls,[7] findings which somewhat disagree with others that show COX-inhibitors do not affect protein synthesis in unstimulated tissue.[16] Nonetheless, these results strongly support the role for COX metabolites in satellite cell activity and add to the growing body of literature to demonstrate prostaglandin antagonists (COX inhibitors) interfere with various stages of the satellite cell cycle.[2, 8, 10-12, 26]

Conclusions

General consensus holds that skeletal muscle regeneration is contingent upon satellite cell activity and protein synthesis,[38] and a plethora of research has substantiated a role for $PGF_{2\alpha}$ in both. As the cell cycle nears completion, myofiber size continues to increase through the processes of myonuclear accretion and the incorporation of amino acids into skeletal muscle.[1, 15, 16, 28] This review has presented research that has demonstrated the detrimental effects of COX-inhibitors on satellite cell activity and mixed muscle protein synthesis in both in vitro and in vivo human and animal research in different models of injury.

While COX-inhibitors are used for a variety of ailments, they remain one of the most popular therapeutic treatments for exercise-associated pain, and non-prescription analgesics are reportedly the most frequently used individual products among FDA-regulated drugs.[42] Exercise-associated pain is often referred to as delayed onset muscle soreness (DOMS). While symptoms initially present 24-hours after novel or intense exercise, they may persist 48-72 hours after exercise-induced muscle injury.[15, 27, 43-49] There are similarities between the onset and persistence DOMS and phases of regeneration occurring in skeletal muscle. For instance, proliferation of in vitro human myoblasts has been reported to peak 24-48 hours after the addition of exogenous $PGF_{2\alpha}$,[2] and inhibition of $PGF_{2\alpha}$ at 24 hours has been shown to be detrimental to muscle cell growth.[1] Mixed skeletal muscle protein synthesis has been reported to be elevated 76% 24-hours after acute resistance exercise in recreationally-trained males,[15] and 64% and 34% at 24- and 48-hours, respectively, after acute resistance exercise in recreationally-active males and females.[31] A different time course has been reported in animal models using alternative techniques to induce injury, with elevations of 83% and 92% above baseline 120 and 72 hours, respectively, after injury.[40, 41] While hypertrophy may be accomplished through either an increase in protein synthesis or a decrease in protein degradation, hypertrophy is usually achieved by an increase in protein synthesis, often with a proportionately smaller increase in degradation.[28] Furthermore, certain non-selective COX-inhibitors (indomethacin) have been reported to exert greater inhibitory effects on $PGF_{2\alpha}$ than PGE.[30] These are important facts, particularly when one considers the ease with which COX-inhibiting drugs can be procured.

In addition to the mechanism of injury, it is also important to consider parameters of the treatment regimen employed in research, including not only the type of COX-inhibitor but also

the onset of administration and the duration of treatment. Indomethacin and aspirin have been reported to significantly inhibit proliferation of human myoblasts when added to culture media when proliferation is at its peak; however, the same COX-inhibitors inhibit fusion when are added close to the onset of differentiation.[2] Fusion has been shown to be most adversely effected when COX-inhibitors are taken 24-hours before the onset of fusion.[2] Thus, according to these findings, analgesics taken for a headache one day before exercise could theoretically inhibit the regenerative response to that exercise bout. Furthermore, chronic low dose administration of indomethacin (non-selective COX-inhibitor) has been shown to be more deleterious to myoblast proliferation than a single, larger dose, holding serious implications for those persons chronically consuming NSAIDs while anticipating muscular fitness gains.[2]

As demonstrated in the only chronic training study to measure $PGF_{2\alpha}$ metabolites, the magnitude of the $PGF_{2\alpha}$ response decreases over 6-weeks of resistance exercise, resulting in no significant differences in strength or skeletal muscle tissue between naproxen-treated and placebo-treated participants (unpublished findings). Thus, the role of $PGF_{2\alpha}$ in skeletal muscle regeneration may be more important in the beginning of a program, when some argue for their use to prevent pain associated with novel exercise,[46, 49, 50] potential injury resulting from compromised contractile abilities post-exercise,[44] or the elevation of protein degradation that occurs with injury.[51]

In conclusion, $PGF_{2\alpha}$ should be recognized as a hormone with effects on skeletal muscle tissue that promote regeneration and ultimately growth. With the majority of research characterized as acute interventions, additional studies examining the effects of chronic administration of COX-inhibitors with the performance of regular exercise on parameters of muscle morphology and function seem warranted. While the adverse effects of COX-inhibitors on the liver and cardiovascular system are well-known, the same may not be said for its effects on skeletal muscle regeneration. Measures to increase awareness of potential consequences of $PGF_{2\alpha}$ suppression should be considered, as these findings are important not only to recreational exercisers and athletes but also to clinicians and patients managing orthopedic injuries and diseases.

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