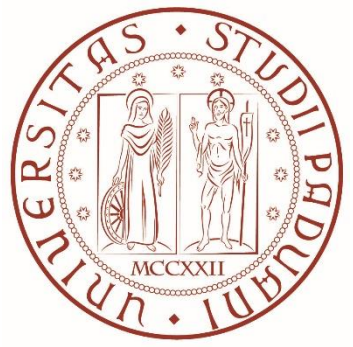

UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartimento di Scienze Biomediche

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Tesi di Laurea

EFFECTS OF NSAIDS ON MUSCLE PERFORMANCE AND EXERCISE RECOVERY

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Contents

Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used by athletes to manage pain and inflammation, and some believe they might help improve performance. However, their actual effects on muscle performance and recovery are not entirely clear. This thesis aims to explore how NSAIDs impact muscle soreness, muscle damage, physical performance, and recovery after different types of exercise.

Chapter 1

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to manage pain and inflammation, especially among athletes and physically active individuals. Their effects on muscle performance, adaptation, and recovery have been extensively studied, yet findings are often nuanced and contradictory. This thesis analyses the results from five selected studies to better understand the implications of NSAID use in the context of exercise and muscle performance. To understand the impact of NSAID use on exercise and muscle performance, it is essential to first briefly explore the underlying inflammation process they are designed to target and their pharmacological action.

1.1 Inflammation

The inflammation process is a defensive reaction of the body that activates the immune system to release inflammatory mediators such as hormones, cytokines, prostanoids, bradykinin and histamine. Inflammation destroys harmful agents and repairs and reconstructs damaged tissue. Inflammation can be acute, "angioflogosis" or chronic, "istoflogosis".
Acute inflammation lasts for a short time and it is characterised by 5 cardinal signs: rubor (redness) because of ongoing dilation of the peripheral blood vessels, allowing more blood to reach the tissue which leads to calor (heat); tumor (swelling); increased endothelial permeability and accumulation of plasma components (exudate), dolor (pain) due to mechanical pressure in the blood vessel and irritation of pain receptors that send signals to the brain in order to make the person protect the affected area and functio laesa (loss of function).
The primary cell involved in acute inflammation is the neutrophil. These are the first responders to the site of injury or infection. They are attracted to the area by chemical signals (chemotaxis) and engulf and destroy invading microorganisms or cellular debris through a process called phagocytosis. Other cells that take part in the inflammation process are: mast cells, macrophages, basophils, eosinophils, fibroblasts, lymphocytes and platelets.
On the other hand, chronic inflammation lasts longer than acute inflammation because the destructive process overpowers the healing process of the tissue. This happens in the case of infections like tuberculosis, autoimmune diseases such as rheumatoid arthritis, lupus, other diseases for example, atherosclerosis, asthma and type 2 diabetes.
The main cells that operate in chronic inflammation are granulomas.

1.2 NSAIDs

NSAIDs, as previously mentioned, are nonsteroidal anti-inflammatory drugs that are used for their analgesic, antipyretic, and anti-inflammatory effects given that they block the formation of key players in the inflammatory response, the prostanoids.
The mechanism of action primarily revolves around their ability to inhibit the cyclooxygenase (COX) enzymes, which are crucial in the biosynthesis of prostaglandins. Prostaglandins are lipid compounds that have various roles in the body, including mediating inflammation, pain, and fever. To better understand the effects of NSAIDs, it is important to differentiate between the two main isoforms of cyclooxygenase enzymes involved in prostaglandin production:

- **COX-1**: This enzyme is constitutively expressed in the body, meaning it is always active in most tissues. By controlling the synthesis of the different prostaglandins and thromboxane, it plays a key role in maintaining normal physiological functions such as protecting the stomach lining, supporting platelet aggregation, and regulating blood flow to the kidneys;

- **COX-2**: This enzyme is inducible, meaning it is produced in response to inflammatory stimuli, such as injury or infection. COX-2 is responsible for the synthesis of prostacyclin.

1.3 Cox Selectivity

Depending on the NSAID, the inhibition can be non-selective (inhibit both COX-1 and COX-2 enzymes) or selective (primarily inhibiting COX-2). Non-selective inhibition of COX-1 and COX-2 can lead to side effects like gastrointestinal irritation and ulcers, as COX-1 normally helps protect the stomach lining (Sohail et al., 2023). Examples of non-selective NSAIDs include ibuprofen, naproxen, and aspirin (Cryer and Feldman, 1998).
Selective COX-2 inhibitors: These drugs specifically target COX-2, with the intention of reducing inflammation and pain while minimising gastrointestinal side effects. However, COX-2 inhibitors can still have other important adverse effects, particularly related to cardiovascular health. Indeed, the inhibition of COX-2 blocks prostacyclin, which normally causes vasodilation and inhibits platelet aggregation, because COX-2 is constitutively present in endothelial cells, where COX-1 is absent. This leads to an imbalance between the reduced presence of prostacyclin and thromboxane, which continues to be produced by COX-1. With thromboxane prevailing, there is increased platelet aggregation and vasoconstriction resulting in a higher probability of thrombus formation and the risk of stroke or cardiovascular events.
Pharmacokinetically, NSAIDs show a lot of variety in terms of absorption, distribution, metabolism, and excretion. Most NSAIDs are rapidly absorbed after oral administration and are metabolised primarily in the liver through the cytochrome P450 enzyme system. They are then excreted by the kidneys. The half-life of NSAIDs varies influencing the duration of action and dosing frequency.

1.4 Therapeutic uses of NSAIDs

NSAIDs are not considered a performance enhancing drug by the World Anti-Doping Agency (WADA, 2024) but they are frequently used by athletes and physically active individuals to manage exercise-induced pain, reduce inflammation, and improve recovery times.
A survey conducted in the US revealed that 75% of 681 student athletes had used NSAIDs in the past three months, with 15% using them daily (Warner et al., 2002).
During the 2014 FIFA (Fédération Internationale de Football Association) World Cup, the most frequently used substance group were NSAIDs, representing 36% of all substances tested (Tscholl PM et al., 2015).
In a marathon study, the analgesics group (49% of participants) had a 13% higher incidence of adverse events (particularly gastrointestinal cramps and cardiovascular events) compared to the control group. Diclofenac (47%) and ibuprofen (43%) were the most used analgesics, often taken at twice the recommended dose (Küster et al., 2013).
A survey conducted in South Africa among triathletes, revealed that 59.9% (196 participants) had used NSAIDs in the preceding three months. Among them, 25.5% took NSAIDs the day before the race, 17.9% took them immediately before the race, and 47.4% used them during the race. Notably, 48.5% of those who used NSAIDs did so without a medical prescription.
The study also revealed that athletes had limited awareness of both the benefits and potential side effects of the medications (Gorski T et al., 2009).
Through a survey, an observational study of competitive mountain bikers found out that of 131 race finishers, 10% used NSAIDs at least once on race day. Only 2% of participants, all from the 24 MTB event, took NSAIDs during the race, and 5% reported using them afterward. NSAID users were generally older, and ibuprofen was the most commonly used drug, taken by 79% of those who used NSAIDs (Chlíbková et al., 2018).
An Italian study identified ibuprofen (13.8%), diclofenac (12.7%), aspirin (7.4%), and naproxen (6.9%) as the most commonly reported NSAIDs used by athletes, along with other NSAIDs like propyphenazone (Mazzarino et al., 2010).
Military personnel use them at the highest rates, with more than 60% of Soldiers filling an NSAID prescription from 2006–2014 in the U.S. army (Walker et al., 2017).
Taken together, NSAIDs are a popular choice for pain management and muscle recovery in several populations.

1.5 Adverse effects

Gastric adverse effects from NSAIDs primarily result from the inhibition of COX-1, which leads to reduced production of prostaglandins that protect the gastric mucosa. This damage is more likely in patients with a history of peptic ulcers. To mitigate these risks, COX-2 selective NSAIDs, which have a lower risk profile, may be a safer alternative.
Renal adverse effects arise because both COX-1 and COX-2 are involved in producing prostaglandins that help maintain renal hemodynamics. In patients with normal renal function, the inhibition of these prostaglandins generally poses less risk. However, in those with renal dysfunction, reduced prostaglandin levels can lead to serious issues such as acute renal dysfunction, fluid and electrolyte imbalances, renal papillary necrosis, and nephrotic syndrome or interstitial nephritis.
Cardiovascular risks associated with NSAID use include myocardial infarction, thromboembolic events, and atrial fibrillation, with diclofenac being particularly linked to a higher incidence of

adverse cardiovascular effects.
Hepatic adverse effects are less common, but NSAIDs can cause hepatotoxicity, such as elevated aminotransferase levels. Although liver-related hospitalizations are rare, diclofenac has been noted for a higher rate of hepatotoxic effects.
Hematologic adverse effects may occur, especially with nonselective NSAIDs due to their antiplatelet activity. These effects are more problematic in patients with a history of gastrointestinal ulcers, conditions affecting platelet function (such as hemophilia or thrombocytopenia), and some perioperative cases.
Other minor adverse effects include anaphylactoid reactions, which can affect the skin and respiratory systems, such as urticaria and aspirin-exacerbated respiratory disease (Goodman and Gilman's, 2022).

1.6 Criteria for the selection of the studies

The studies discussed in the present thesis were selected based on publication date and if they were double blind or not.
Key words such as: NSAIDs, muscle soreness, exercise, anti inflammatory drugs, muscle recovery, sport performance, muscle signalling and satellite cells, were used. Databases such as: PubMed and Google Scholar were used.

1.7 Aim of the thesis

The aim of the thesis is to understand the relation between NSAIDs and muscle performance, analysing: sport performance, muscle soreness, muscle signalling, dosage of NSAIDs, endurance training and mitochondrial function.

Chapter 2

Literature review

2.1 NSAIDs do not prevent exercise-induced performance deficits or alleviate muscle soreness: A placebo-controlled randomized, double-blinded, cross-over study

Brandon M Roberts, Cara E Sczuroski, Aaron R Caldwell, David J Zeppetelli, Nathaniel I Smith, Vincent P Pecorelli, Jess A Gwin, Julie M Hughes, Jeffery S Staab
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Introduction

NSAIDs are commonly used by athletes to reduce soreness and speed up recovery after exercise. Studies show that around 25% of female and 20% of male collegiate athletes, as well as 36% of recreationally trained students, report using NSAIDs for pain or prevention. The effects of NSAIDs on muscle soreness and performance vary based on the type, dose, and timing. Some studies show that NSAIDs can reduce soreness and improve performance, while others do not find a significant impact.

Objective

This study aims to compare the effects of flurbiprofen, a primarily COX-1 inhibitor, celecoxib, a COX-2 inhibitor and ibuprofen, a non-selective COX inhibitor, on muscle soreness and performance after a plyometric training session, hypothesising that all NSAIDs would reduce muscle soreness and improve performance compared to a placebo.

Methods

Twelve healthy adults (10 men and 2 women, ages 18–42) who exercised at least twice a week participated. The study included a baseline week followed by four experimental trials, each separated by a minimum of seven days. Participants completed familiarisation and baseline assessments prior to the experimental trials.

Muscle soreness, maximum voluntary contraction force (MVC), heart rate, perceived exertion (RPE), and vertical jump height were measured at baseline, pre-exercise, and 4 and 24 hours post-exercise. The study used a Plyo-press, a modified leg press machine, to provide the exercise stimulus and measure both one-repetition maximum (1RM) and MVC. Participants lay on a sled that moved along a track as they pushed off a stationary platform.

MVC was recorded after a warm up, with participants pressing maximally for 5 seconds while maintaining 90° knee and hip angles. The highest of two trials was recorded. The 1RM was determined by progressively adding weight until the participant could no longer successfully complete a leg press. This measurement was only taken during baseline testing to set the appropriate weight for subsequent exercise trials.
For the vertical jump: participants performed two warm up jumps before attempting three jumps and the highest score was used.
In each of the four study trials, participants performed a single bout of plyometric exercise at 40% of their 1RM on the Plyo-press. This exercise included 10 sets of 10 jumps, with each jump phase occurring at a rate of about 1 Hz and a two-minute rest between sets.
Heart rate was monitored and recorded after each set, while perceived exertion was measured using the Borg Scale. Power output was tracked using a force plate attached to the Plyo-press.
As for muscle soreness, a 10 cm visual analog scale was used to measure it in four body regions (shoulder, quadriceps, gluteus maximus, and calves). Participants rated soreness from 0 (no soreness) to 10 cm (maximal soreness) by marking a line with a pen.
Participants arrived after an overnight fast for weigh-ins and a soreness survey, followed by the administration of either 800mg ibuprofen, 200mg celecoxib, 100mg flurbiprofen or a placebo. The dosage timing and exercise start times were consistent for each trial, varying by no more than ±30 minutes. Both participants and the investigative team were blinded to the treatments. After NSAID ingestion, participants ate a standardised light breakfast. Pre-exercise tests (MVC and vertical jump) were conducted, followed by a plyometric exercise bout 2 hours later. Post-exercise MVC, vertical jump tests, and soreness surveys were repeated 4 and 24 hours afterward.
Data were checked for normality and analysed using linear mixed models and one-way ANOVA. Significance was set at $p < 0.05$, with results reported as Mean ± SD.

Results

There were no significant differences between ibuprofen, celecoxib, flurbiprofen or placebo use in mean work, heart rate, or RPE during the plyometric exercise sessions, indicating no treatment affected exercise intensity or workload. While there was no treatment by time effect for soreness in the shoulder, quadriceps, gluteus maximus, or calves, there was a significant time effect for all muscle groups, indicating that plyometric exercise caused soreness but NSAIDs did not reduce it 4 or 24 hours post-exercise. Vertical jump performance was consistent across trials, with no treatment by time effect, though a significant time effect was observed. There was a treatment by time effect for MVC, with significant differences pre-exercise between ibuprofen and flurbiprofen, and post-exercise between placebo and celecoxib where with both NSAID treatments, increased MVC was recorded.

Discussion

This study is the first to compare NSAIDs with three different COX affinities (celecoxib targets COX-2, flurbiprofen COX-1 and ibuprofen is non-selective for COX-1 and -2) to determine if they play a role in reducing muscle soreness or preventing reductions in performance.
The NSAID dose used in this study was the highest recommended single dose, and timing was chosen to have peak levels of NSAID in participants blood during exercise.
A study by Hasson et al., (1993) found that taking 400 mg of ibuprofen 4 hours before eccentric stepping exercise reduced muscle soreness 24-48 hours post-exercise. However, Donnelly et al.,

(1990) observed that a 1200 mg dose of ibuprofen taken 30 minutes before downhill running had no effect on muscle soreness. Similarly, Krentz et al., (2008) reported that taking 400 mg of ibuprofen after repeated resistance training sessions for 6 weeks did not affect muscle soreness, and Rahnama et al., (2007) found that 400 mg of ibuprofen taken before and after eccentric exercise on 7 occasions did not reduce soreness. Furthermore, de Souza et al., (2020) found that 400 mg of ibuprofen taken before and after a 42 km running competition did not reduce muscle damage.

In terms of performance, Cornu et al., (2020) in its meta-analysis found low-quality evidence and inconsistent NSAID dosing across studies, making it difficult to conclude whether NSAIDs enhance sports performance.

As for celecoxib, this study found that 200 mg of celecoxib reduced muscle strength loss 4 hours post-exercise but did not affect muscle soreness. However, a separate study using 400 mg of celecoxib did show reduced muscle soreness in the arms after exercise (Paulsen et al., 2009).

The findings from Roberts et al., (2024a) align with previous research, such as Tokmakidis et al., (2003), which showed that ibuprofen did not improve vertical jump performance post-exercise. Collectively, the evidence suggests that NSAIDs generally do not enhance physical performance.

Conclusion

Exercise often leads to muscle soreness, which may negatively impact performance. Athletes may turn to NSAIDs to alleviate soreness and preserve performance. This study found that a high prophylactic dose of ibuprofen or flurbiprofen taken before intense plyometric exercise had no effect on muscle soreness, RPE, HR, MVC, or vertical jump performance compared to a placebo. However, celecoxib demonstrated an improvement in short-term performance as measured by MVC. Although some researchers have found that NSAIDS might impact sport performance and muscle soreness, there is still wide controversy surrounding this topic.

2.2 A single, maximal dose of celecoxib, ibuprofen, or flurbiprofen does not reduce the muscle signalling response to plyometric exercise in young healthy adults

Brandon M. Roberts, Alyssa V. Geddis, Cara E. Sczuroski1, Marinaliz Reynoso, Julie M. Hughes, Jess A. Gwin, Jeffrey S. Staab (2024)
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Introduction

Skeletal muscle protein synthesis during post-exercise recovery is critical for muscle growth, but NSAIDs may impair this process by inhibiting COX enzymes involved in inflammation.
This has been shown to hinder muscle regeneration and synthesis in both rodents (Soltow et al., 2006) and humans (Trappe et al., 2002). Muscle protein synthesis, which is essential for muscle growth and repair, is regulated by key signalling pathways such as mTOR and ERK.
These pathways are activated post-exercise and play a crucial role in translation initiation and muscle mass gains over time. Prostaglandins, especially PGF2α, are known to stimulate mTOR signalling and promote muscle hypertrophy, with their levels rising during post-exercise recovery. However, NSAIDs can inhibit this increase in prostaglandins.
There is conflicting evidence on the impact of NSAIDs on muscle adaptation and exercise-related signalling. Some studies report that NSAIDs may reduce muscle protein synthesis (MPS) signalling through the AKT-mTOR pathway, alter satellite cell signalling, decrease muscle protein degradation, and lower cell proliferation.
Also, based on previous research, the hypothesis was that ibuprofen, but not celecoxib or flurbiprofen, would attenuate the MPS signalling response induced by exercise (Lilja et al., 2018; Markworth et al., 2014; Trappe et al., 2002; Burd et al., 2010).

Objective

In this study, it was investigated whether a single maximal dose of flurbiprofen (FLU), celecoxib (CEL), ibuprofen (IBU), or a placebo (PLA) influences short-term muscle signalling responses following plyometric exercise.

Methods

This was a block randomised, double-masked, crossover design, where 12 participants (10 males and 2 females) ages 18-42 years old who exercised at least twice a week, performed four plyometric exercise bouts consisting of 10 sets of 10 plyometric jumps at 40% 1RM. Two hours before exercise, participants consumed a single dose of celecoxib (CEL 200 mg), IBU (800 mg), FLU (100 mg) or PLA (placebo) with food. Muscle biopsy samples were collected before and 3-h after exercise from the vastus lateralis. The investigation assessed muscle tissue markers for COX, ribosomal, satellite cell, as well as protein degradation and protein synthesis signalling. The quad muscle was homogenised, and RNA was extracted. Real-Time PCR was performed on cDNA.
Protein levels of the post-exercise biopsy were normalised to its respective pre-exercise biopsy sample. Data were analysed using repeated measures (RM) ANOVA, ANOVA, or a Friedman test. Significance was considered at $p<0.05$.

Results

There were no differences between treatments for COX1 and COX2, represented by PTSG1 and PTSG2 respectively.

The data indicate that muscle signalling related to ribosome biogenesis was not affected by NSAIDs, measured via total RNA, mRNA expression, and protein abundance.

To determine if protein degradation signalling was influenced by NSAID treatment: MURF1 and Atrogin-1/MAFbx were measured. No treatment effect for both were found. Satellite cell signalling was measured by expression of PAX7, MYOD1, and MYOG, but no treatment effect was found. Furthermore, the study found no treatment effect for AKT/mTOR, and also for RPS6.

The researchers evaluated how NSAIDs affect the MAPK signalling pathway by measuring the levels of phosphorylated (activated) ERK, p38, and MNK1. While the treatments did not significantly affect the activation of p38 and ERK proteins, they did have a significant impact on the activation of MNK1, particularly when comparing the placebo to flurbiprofen treatment.

Discussion

After investigating the effects of a single, maximum over-the counter dose of celecoxib, ibuprofen, flurbiprofen, or placebo on post-exercise skeletal muscle signalling, no differences were found between treatments post-exercise.

The current study suggests that the presence of an NSAID does not blunt COX-2 expression or quantity in skeletal muscle. Other data is in agreement with this study, indicating that COX-2 expression is increased 3–4 h post-exercise (Roberts et al., 2024b).

Researchers also speculate that COX-2 plays a role in MPS, since it is increased with training (Lundberg and Howatson 2018).

In support of this, experiments suggest COX-2 specific NSAIDs interfere with growth and regeneration in vitro (Matheny et al., 2022).

But some scientists have speculated that COX-1 might be involved in the post-exercise increase in MPS, suggesting that the COX-1 isoform may be responsible for the COX-mediated postexercise increase in muscle protein synthesis in humans and this explanation aligns with the idea that COX-1 is a constitutively expressed protein involved in maintaining normal cellular functions, such as protein synthesis in skeletal muscle cells (Burd et al., 2010).

Previous studies have found NSAIDs influence both translational signalling and muscle protein signalling in young adults (Markworth et al., 2014; Trappe et al., 2002; Burd et al., 2010).

Markworth et al., (2014) found that ibuprofen inhibits MEK-ERK signalling in human muscle during post-exercise recovery, leading to reduced phosphorylation of p70S6K and rpS6. This finding contrasts with the study by Roberts et al., (2024b). However, Markworth et al., (2014) also observed that MNK1 levels were lower in the ibuprofen group compared to the placebo group, a result that aligns with Roberts et al., (2024b), following participants' completion of 3 sets of 8-10 repetitions of lower body exercises at 80% intensity.

Differences in findings between the two studies could be due the divergences in training volume and intensity.

Other research conducted on participants after four weeks of flywheel training found that ibuprofen (400mg before exercise, 12000mg daily, during 8 weeks) does not attenuate the p-mTOR, p-p70S6K, or p-ERK1/2 responses 3 hours post-exercise (Lilja et al., 2018), and is consistent with findings from Roberts et al., (2024b).

NSAID timing and dose may also play a role in translational signalling: Lilja et al., (2018)

found that ibuprofen attenuated increases in muscle volume with 8 weeks of flywheel training, Trappe et al., (2002) also gave a single 400 mg dose of ibuprofen at the start of the exercise protocol and found MPS was blunted.
However, since the current study used a significantly larger dose of ibuprofen (800 mg) compared to the doses used in the studies by Trappe et al., (2002) and Lilja et al., (2018), the observed differences are likely due to the varying exercise stimuli and measurement durations in those studies. Overall, current literature suggests that the effect of NSAIDs on muscle protein synthesis signalling remains uncertain and is more likely influenced by the exercise stimulus rather than the specific NSAID dose (Roberts et al., 2024b).
NSAIDs do not significantly impact ribosome signalling during the acute exercise response, indicating their effect on muscle protein synthesis is likely minimal and primarily related to translational efficiency rather than overall capacity (Roberts et al., 2024b).
In this study, NSAIDs had no impact on satellite cell signalling. Previous research shows mixed results: some studies found that NSAIDs, like indomethacin, can reduce satellite cell proliferation post-exercise (Mackey et al., 2007; Mikkelsen et al., 2009), while others, such as Mackey et al., (2016), observed that ibuprofen can speed up satellite cell activation after muscle injury. The lack of findings from the current study may be due to the measurements being taken only 3 hours post-exercise, unlike other studies that measured at later recovery stages.
However, ibuprofen ingestion by healthy elderly individuals participating in a 12-wk program of resistance training has been shown to reduce the increase in the muscle gene expression levels of the cytokines interleukin-6 and -10 observed in the placebo group and promote muscle growth (Trappe et al., 2013).

Conclusion

In conclusion, the study found that three NSAIDs with varying COX selectivity, when taken two hours before exercise, did not significantly affect acute muscle protein synthesis signalling or satellite cell responses three hours post-exercise. Although flurbiprofen reduced MNK1 activation, this reduction was not significant enough to negatively impact muscle signalling.
Variations in findings across studies may be attributed to differences in exercise protocols, NSAID doses, and timing of measurements.

2.3 The dose-response effects of flurbiprofen, indomethacin, ibuprofen, and naproxen on primary skeletal muscle cells

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Introduction

Previous research suggests that NSAIDs can interfere with muscle protein synthesis and satellite cell proliferation after exercise. This study adds information regarding the effect of ibuprofen, flurbiprofen, naproxen sodium, and indomethacin on exercise by recreating exercise stimuli in vitro. They are all non-selective NSAIDs, but naproxen sodium is stronger compared to the others, with indomethacin and flurbiprofen showing greater selectivity for COX-1.

Objective

This study investigates the effect of ibuprofen, flurbiprofen, naproxen sodium, and indomethacin at different concentrations on myoblast proliferation, myotube area and fusion, prostaglandin production, and muscle protein synthesis pathways, especially using indomethacin.

Methods

Myoblasts and myotubes were grown and expanded in Skeletal Muscle Cell Growth Media and Bullet Kit. ELISA kits were used for PGE2 and PGF2α.
Cellular protein extraction and immunoblotting were conducted.
Statistical analyses were performed using a one-way analysis of variance (ANOVA).
A p-value of < 0.05 was considered significant.

Results

Firstly, the study tested the effects of ibuprofen, flurbiprofen, indomethacin, and naproxen sodium on myoblast proliferation (indirect measure for satellite cell proliferation) over 48 hours. Ibuprofen showed no significant impact across $25\mu M$, $50\mu M$, $100\mu M$, and $200\mu M$ concentrations. Flurbiprofen at low concentrations ($25\mu M$) increased proliferation and high concentrations ($200\mu M$) decreased it. Indomethacin reduced proliferation at concentrations $100\mu M$ and $200\mu M$, while naproxen sodium increased proliferation at $25\mu M$ concentration but had no effect at higher concentrations.
Secondly, the study examined the effects of different NSAIDs on myotube area and fusion, which more closely reflects how these drugs might affect muscle changes in humans. Flurbiprofen increased myotube area at a $50\mu M$ concentration but did not affect fusion. Ibuprofen had no significant impact on either myotube area or fusion at any of the tested concentrations.
Indomethacin reduced myotube fusion at high concentrations of $200\mu M$ while naproxen sodium had no significant effects on either myotube area or fusion at any concentration.
Next, the study assessed how different concentrations of indomethacin (0.0125, 0.025, 0.05, 0.10, 0.20, 0.40, 0.80, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, and 200 μM) would affect prostaglandin (PG) production. Primary human skeletal myoblasts were treated with arachidonic acid (AA) to mimic the short-term muscle cell response that occurs post-exercise, and with varying

concentrations of indomethacin for 48 hours. The results showed, as expected, that AA increased production of PGE2 and PGF2α. Indomethacin significantly reduced the AA-induced PGF2α response at all tested concentrations, but only decreased the PGE2 from $0.10\mu M$ to $1.56\mu M$, and from $12.5\mu M$ to $200\mu M$. Lastly, the study investigated more deeply the impact of indomethacin on muscle protein synthesis pathways. Statistical analysis revealed that indomethacin at $200\mu M$ significantly decreased the ratio of phospho-P70 to total P70 by $62 \pm 4\%$ ($p < 0.001$), but had no significant effects on the AKT pathway. These findings suggest that high concentrations of indomethacin negatively impact muscle protein synthesis, potentially explaining the observed reduction in myotube fusion.

Discussion

The study examined how various NSAIDs affect muscle cells, specifically myoblast proliferation, myotube size, and fusion. High concentrations of indomethacin ($200\mu M$) reduced prostaglandin production and impaired protein synthesis pathways, which likely reduced myotube fusion and inhibited myoblast proliferation.

On the other hand, ibuprofen and naproxen sodium had no significant effects on these aspects, while flurbiprofen showed a dose-dependent response: it increased proliferation at a low concentration ($25\mu M$) but decreased it at a high concentration ($200\mu M$).

The circulating half-life varies among the NSAIDs: ibuprofen is three to four hours, flurbiprofen is three to six hours, indomethacin is five to ten hours, and naproxen sodium is twelve to eighteen hours (Roberts et al., 2024c). These half-lives guide dosing frequency and influence the concentration levels in human plasma.

The NSAIDs administered in this study reach their peak concentration in blood plasma at around two hours after ingestion.

The concentrations found in human blood plasma for naproxen sodium at a 440 mg dose (the typical dosage is 220 mg) peak at $60\mu g/mL$, which is comparable to a $200\mu M$ concentration. Additionally, its decay to $5\mu g/mL$ by 72 hours is similar to a $25\mu M$ concentration at 24 hours (Roberts et al., 2024c). Consumption of a single 600 mg dose of ibuprofen was $29\mu g/mL$. Translating the information to the current study, it would represent the $\sim 200\mu M$ of ibuprofen at its peak (Roberts et al., 2024c). In human plasma, 100 mg of flurbiprofen causes mean peak concentration of 42.1 mg/mL after 1.5h and decays to $\sim 10\mu g/mL$ eight hours later (Yilmaz et al., 2015), which is comparable to the concentrations of $200\mu M$ and $50\mu M$ used in cells, respectively. As for indomethacin, the peak human concentration on a 50 mg dose is comparable to a $\sim 12.5\mu M$ concentration in vitro although the normal dose of 25 mg produces concentrations of $\sim 2.5\mu g/mL$ in blood plasma (Roberts et al., 2024).

By testing lower concentrations of indomethacin, which are still double those found in human plasma, the study observed no impact on myogenesis at $25\mu M$. Thus, it is unlikely that humans who take low to moderate doses of indomethacin will have an effect on muscle cells.

Studies using indomethacin to inhibit prostaglandin synthesis have also reported a reduction in myotube fusion (Zalin, 1977). This further supports the involvement of the COX-1 pathway in maintaining muscle cell homeostasis. Then again, it appears that a 45 mg dose of indomethacin does not inhibit the MPS response (Mikkelsen et al., 2011).

Previous research has indicated that ibuprofen inhibits the MPS response after exercise in humans, but not proliferation in muscle cells (Trappe et al., 2002; Markworth et al., 2014). However, some researchers consider this topic to be rather uncertain (Roberts et al., 2024c).

Conclusion

In summary, the research found that high concentrations of indomethacin ($200\mu M$) inhibited myoblast proliferation and disrupted protein synthesis signalling in myotubes. In contrast, ibuprofen and naproxen had no significant effects, while flurbiprofen increased myoblast proliferation at low concentrations but decreased it at higher concentrations.

The study provides new insights into how NSAIDs influence muscle cells in vitro.

2.4 Effects of ibuprofen during 42-km trail running on oxidative stress, muscle fatigue, muscle damage and performance: a randomized controlled trial

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Introduction

Trail running involves challenging terrain that leads to extensive muscle activity, especially in the eccentric phase, which causes muscle damage, oxidative stress (OS), and inflammation.
OS from prolonged, intense exercise, increases oxygen consumption and reactive oxygen species (ROS) production, this makes ultramarathoners more susceptible to oxidative diseases (Mrakic-Sposta et al., 2015).
Muscle damage was measured indirectly using creatine kinase (CK) levels, since when muscle cells are damaged or stressed, such as during intense exercise or in conditions like muscle injuries or heart attacks, CK levels in the blood increase.
To manage the pain and the inflammation, many trail runners use nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, which is widely available and believed to reduce pain and muscle damage. In fact, it is estimated that up to 75% of ultramarathon runners consume ibuprofen (Lipman et al., 2017).

Objective

This study aimed to investigate the impact of prophylactic use of IBU on markers of oxidative stress, muscle damage, vertical jump and running performance of a 42 km trail run (TR).

Methods

A randomised control trial was conducted at the 42km challenge in Brazil with 12 male runners divided into two groups: ibuprofen (n=6) and placebo (n=6). The study aimed to assess the effects of prophylactic ibuprofen use on performance, oxidative stress, and muscle damage during a 42 km trail race. Participants met criteria including a half-marathon time of 1:45–2:00 hours and no recent muscle, joint, or heart issues.
In the study, athletes in the ibuprofen group (IBG) took a 400 mg ibuprofen capsule 15 minutes before the race and another after 5 hours of running. Blood samples were collected from all participants before and after the race to measure markers of muscle damage and oxidative stress (OS). Performance was assessed through squat jump (SJ), countermovement jump (CMJ), total race time, speed, and pace, with pace monitored using chips attached to the runners' shoes.
The quantification of the tissue lesion caused by the 42-km TR test was determined by the enzyme creatine kinase (CK) and the quantification of OS was determined by the TBARS (Thiobarbituric Acid Reactive Substances) lipid oxidation marker.
For the evaluation of the SJ and CMJ tests, a 50 $\times$ 60 cm conductive surface contact mat was used to measure the height of the vertical jump, which was determined by the time interval between the feet losing contact with the mat and their subsequent contact upon landing.
A statistically significant difference was adopted between the samples for $p < 0.05$.

Results

The study found a significant reduction in squat jump performance, with a 44% decrease from baseline in the placebo group compared to the - 36% in the ibuprofen group (IBG). However, no significant differences were noted in countermovement jump, pace, speed, or finish time between the groups. Oxidative stress markers increased by 70.1% in the placebo group after the race, along with a 40.1% reduction in the antioxidant defence system (glutathione-SH). In contrast, the IBG group showed a 31.41% increase in glutathione activity in comparison to the placebo. An increase in the CK marker for muscle damage was found in both the placebo group and the IBG, but the IBG had a lower increase compared to the placebo group.

Discussion

Key findings showed that while IBU reduced oxidative stress and improved SJ performance, it had no effect on overall physical performance, including pace, speed, or running time.

The usage of NSAIDs inhibits the oxidative cascade of arachidonic acid, reducing the expression of proinflammatory enzymes (Rainsford, 2009) and lowering the production of OS and ROS. Although IBU did reduce OS production in comparison to the placebo and did maintain a higher endogenous antioxidant defence system, it didn't have any positive effect on performance. Therefore, it appears that OS results do not relate to the performance results.

Other studies evaluating athletes' performance after using ibuprofen during strenuous exercise have produced conflicting and inconclusive results (Da Silva et al., 2011, de Souza et al., 2020). Similarly to the current study, de Souza et al., (2020) found that ibuprofen taken before a 24 km trail run didn't attenuate muscle damage.

Furthermore, prophylactic administration of 1.2g of ibuprofen had no improvement on creatine kinase, oxygen consumption, pain, vertical jumps, and exhaustive running tests after participants underwent a lower muscle damage induction protocol with an isokinetic dynamometer, using eccentric and concentric combined exercise (Da silva et al. 2015).

In contrast with de Souza et al., (2024) who found a reduction in OS in the IBU group, Mcanulty et al., (2007) found an increase in OS (measured through urinary F2-isoprostanes) after IBU use, but without any improvement in athletic performance after a 160 km run.

Conclusion

In conclusion, this study found that while ibuprofen reduced oxidative stress and improved squat jump in comparison to the placebo group after a 42 km trail run, it did not enhance overall physical performance, including pace, speed, or running time. The reduction in oxidative stress did not translate to better athletic outcomes.

2.5 Resistance Training with Co-ingestion of Anti-inflammatory Drugs Attenuates Mitochondrial Function

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Introduction

The effects of aerobic exercise on mitochondrial content and function are well-documented, but the impact of resistance training remains less clear.
While resistance exercise can stimulate mitochondrial protein synthesis in untrained individuals, early research suggested a reduction in mitochondrial content due to muscle hypertrophy. Conflicting findings exist, with some studies reporting decreased or unchanged levels of oxidative enzymes after resistance training, though recent evidence suggests potential improvements in mitochondrial respiratory function. Nonsteroidal anti-inflammatory drugs (NSAIDs), commonly used by athletes, may interfere with muscle protein metabolism and mitochondrial adaptations. Research has shown that NSAIDs might impair mitochondrial metabolism and reduce ATP production by disrupting mitochondrial function, particularly affecting β-oxidation and mitochondrial respiration in rats (Cardinale et al., 2017). Despite these results, a study on elderly individuals found that NSAIDs increased mitochondrial enzyme activity and muscle mass during resistance training (Trappe et al., 2016).

Objective

This study aimed to investigate the effects of high vs. low doses of NSAIDs on mitochondrial adaptations during an 8-week resistance training program. The researchers hypothesised that high NSAID doses would impair both mitochondrial content and function, while low doses might have less impact.
Additionally, the study compared eccentric overload with conventional resistance training.

Methods

This study was a single-blind randomised controlled training study with parallel groups.
Twenty moderately active men and women participants were randomly assigned to one of two groups for an 8-week supervised knee extensor resistance training program. One group took high doses of ibuprofen (IBU; 3 $\times$ 400 mg/day, age 27 $\pm$ 5 years; n = 11), while the other group took a low dose of acetylsalicylic acid (ASA; 1 $\times$ 75 mg/day, age 26 $\pm$ 4 years; n = 9). Each participant's legs were randomly assigned to train using either a flywheel (FW) device for eccentric overload or a traditional weight stack (WS) machine. FW allows for maximum voluntary force to be produced from the very first rep, while in WS training, full strength is reached only in the last few reps, closer to failure. The 8-week training program focused on the knee extensor muscles and included 20 sessions held 2-3 times per week. Each session began with a standardised warm-up: 2 sets of 5 reps per leg in the FW (flywheel) and 1 set of 5 reps in the WS (weight stack). After a 2-minute rest, participants completed 4 sets of 7 maximal reps in the FW using the leg designated as the "FW leg," followed by 4 sets of 8-12 reps to failure in the WS with the "WS leg." The order of machines alternated between sessions.
Maximal mitochondrial oxidative phosphorylation (CI+IIP) in permeabilized skeletal muscle bundles was measured via high-resolution respirometry. Muscle biopsy samples were taken

from the middle portion of the vastus lateralis from the right leg before training (PRE) and from each leg 48h after the last training session (POST). Citrate synthase (CS) activity was evaluated using spectrophotometry, and mitochondrial protein content was assessed through western blotting.
Muscle quadriceps femoris volume was measured by magnetic resonance imaging (MRI).
The researchers gave the control group a low dose of ASA to eliminate the influence of COX inhibition in platelets as a variable, so they could focus on other effects of the drugs. This way, the COX inhibition in platelets, which happens with both ibuprofen and aspirin, would not affect the study's outcomes.

Results

Both legs experienced a decrease in CI+IIP, but the reduction was significantly greater in the traditional WS machine (33%), compared to the FW device (19%). No differences were observed between the different NSAID. Additionally, no effects of the drug or leg were found when analysing other mitochondrial respiration parameters such as Electron-transferring flavoprotein oxidative phosphorylation complex (ETFP), Electron-transferring flavoprotein Leak respiration (EFTL), chaperone Interaction Protein or Carboxyl-terminal Interacting Protein (CIP), and uncoupling state (Unc). However, significant effects of time were observed, with both ETFP and CIP showing a decrease.
When mitochondrial respiration was adjusted for citrate synthase (CS) activity, ETFP decreased with resistance training. There was also a trend toward decreased CIP and CI+IIP. Additionally, CIP showed an interaction between leg and time, with the decrease being more pronounced in the FW leg. CS activity increased with training with no differences in treatments.
As for protein expression, no interactions were found between groups or legs for various protein levels. However, LC3B-II and the LC3B-II/LC3B-I ratio showed changes depending on the group, with a decrease in ASA and stable or increased levels in IBU. Total-ULK1 increased by about 60% in both groups, while the phospho-ULK1/total-ULK1 ratio decreased. There was also a trend toward increased Beclin-1 with training.
The average muscle volume increased by 4.8%, however, when looking at the relationship between this increase in muscle volume and changes in the CI+IIP (a measure related to mitochondrial function), no correlation was found ($R = -0.16$, $p = 0.33$).

Discussion

This study investigated the impact of resistance training combined with high or low doses of NSAIDs on mitochondrial function and content. Contrary to the researchers expectations, 8 weeks of resistance training led to reduced mitochondrial function but increased mitochondrial content, regardless of NSAID dosage.
So, while this study found reduced mitochondrial function, another study by Porter et al., (2015), reported enhanced mitochondrial respiration after 12 weeks of resistance training. In that study, participants performed upper and lower body exercises, focusing on leg muscles with 3–4 sets of 8–10 reps at 60–80% of their 1RM, three times per week for 70–90 minutes. The differences in findings between Porter's study and the current one may be due to the longer training duration and higher volume, in addition to the use of NSAIDs in the current study.
Secondly, Cardinale et al., (2017) found increased citrate synthase (CS) activity that indicated higher mitochondrial content, but no corresponding changes in mitochondrial protein complexes

were observed. Additionally, mitochondrial function decline was not linked to quadriceps muscle volume changes.
Thirdly, this study observed that high doses of NSAIDs (IBU 1200 mg total) did not have a more negative effect on mitochondrial function compared to low doses of ASA 75 mg, indicating that resistance training was the main driver of this effect, rather than the NSAIDs. The research also explored the role of mitophagy and autophagy markers.
A key autophagy marker, total-ULK1, was significantly upregulated after training, but the level of phospho-ULK1 at Ser317 was lower relative to total-ULK1. To see if mitophagy signalling was upregulated with exercise, different proteins involved in the lysosome formation were measured (i.e., Beclin-1, LCB3-I, and LCB3-II), but no changes in these proteins were found except for upregulation of Beclin-1, hinting that the cells were trying to enhance the removal and recycling of dysfunctional mitochondria. However, since mitochondrial function decreased with training, and simultaneously mitochondrial content increased, impaired mitochondria may have accumulated and therefore caused the increased mitochondrial content.
The rate of mitochondrial ROS (reactive oxygen species) emission decreases as respiration increases. Therefore, high levels of respiration should reduce ROS production (Picard et al., 2011), making it unlikely that excessive ROS caused by high oxygen flux was behind the observed mitochondrial dysfunction. Instead, the dysfunction is more likely due to inflammation from muscle damage during resistance exercise, even with NSAID treatment.
The results suggest that FW resistance training, which emphasises eccentric overload, may help preserve mitochondrial function better than conventional WS training. The exact reason is unclear, but it is speculated that FW training triggers a stronger inflammatory response, which, over time, reduces overall stress and ROS production, potentially explaining the improved mitochondrial function with FW training.

Conclusion

In conclusion, the study demonstrates that 8 weeks of resistance training, combined with both high and low doses of NSAIDs, reduced mitochondrial function but not mitochondrial content in healthy young individuals. Notably, the reduction in mitochondrial function from resistance exercise was not aggravated by higher NSAID doses, suggesting that the training itself was the primary factor influencing this effect. Additionally, the findings indicate that flywheel resistance training, which emphasises eccentric overload, may help counteract some of the decline in mitochondrial function observed with conventional resistance training.

Chapter 3

Discussion and conclusion

This thesis aims to improve our understanding of how NSAIDs affect muscle performance by analysing various factors that could influence outcomes, including muscle soreness, muscle signalling, satellite cell activation, different dosages and type of NSAIDs, endurance sports performance, and mitochondrial function.
Five studies were selected from the numerous papers examining NSAIDs and muscle performance, with each study undergoing a more in-depth analysis focused on specific aspects of muscle performance. The evidence suggests that NSAIDs generally offer little benefit in reducing muscle soreness or improving performance (Roberts et al., 2024a). Among the reviewed studies, it was observed that NSAIDs might influence muscle performance based on factors such as training type, NSAID used, and dosage. However, despite the different analysed factors, the experimental data did not show significant differences in the sport performance.
The impact on muscle signalling and mitochondrial function varies across studies. While most studies that observed the AKT-mTOR pathway found that muscle signalling was unaffected by NSAIDs (Roberts et al., 2024b), mitochondrial function was often reduced after treatment (Cardinale et al., 2017). Notably, although flurbiprofen apparently reduced MNK1 activation, this reduction was not significant enough to negatively impact muscle signalling (Roberts et al., 2024b). High concentrations of NSAIDs, such as indomethacin at 200 µM, can impair muscle cell function (Roberts et al., 2024c). However, achieving such levels in the bloodstream is unrealistic since a typical 50 mg dose of indomethacin results in only $\sim 12.5\mu M$ in vivo.
In endurance contexts, ibuprofen may reduce markers of muscle damage but does not enhance overall performance (de Souza et al., 2024). Interestingly, eccentric overload training, such as flywheel resistance training, may better preserve mitochondrial function compared to conventional training, potentially due to differences in inflammatory responses (Cardinale et al., 2017). However, the role of NSAIDs in these contexts remains unclear.
Additionally, many exercise protocols used in the studies involved untrained participants and were quite intense. Since most beginners typically do not focus on eccentric and plyometric movements, the results may not fully reflect real-world training scenarios. Moreover, flywheel machines, commonly used in research, are not typically available in regular gyms, further limiting the applicability of the results. In summary, the research indicates that the impact of NSAIDs on skeletal muscle varies depending on dosage, COX selectivity, and the type or intensity of exercise.
This thesis also highlights several limitations in the available research. The quality of the studies could be improved, as some data were difficult to interpret, being reported only in

graphs without clear explanations. Contradictions within the same study for example concerning Roberts et al., (2024c), also complicated the analysis. Plus, when analysing the article, the original citations were checked to ensure the accuracy of the reported information. However, in some instances, such as in the case of Roberts et al., (2024b) and Roberts et al. (2024c), the cited information could not be found.

Taking everything into account, further research is needed to clarify these effects and improve our understanding of how NSAIDs affect exercise and muscle health. Additionally, exploring the long-term consequences of chronic NSAIDs use on exercise performance, muscle growth recovery, soreness, muscle signalling, and adaptability to exercise would be valuable.

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