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Prescription Strength Ibuprofen Interferes with
Prophylactic Adaptations to Heavy Exercise

Jessica G. Ehrbar

A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

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Dr. Kent Todd

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A special thank you to the interns and graduate students who directly helped with my project. I literally could not have lifted the weight without you and for that I am much obliged.

Lastly, a thank you to the most important people in my life: my family and Andrew D'Lugos for their unending support, and encouragement throughout the many seasons of my project. Andrew, you will forever be my role model and have led me to become the researcher I am today.

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Abstract

PURPOSE: This proof of concept study was designed to assess the influence of prescription-strength Ibuprofen (IBU) on the repeated bout effect (RBE) of heavy exercise. **METHODS:** Eight males (23 ± 4 yrs) with no recent history of lower-body resistance training completed two separate testing phases separated by a seven-day washout period. Each phase consisted of two sessions of single-legged resistance exercise (RE), performed on the same leg, separated by ten days. One RE trial included 10 sets of 10 repetitions of maximal unilateral eccentric leg extensions at 120% 1RM. Each phase was characterized by a distinct treatment of either 800mg IBU or placebo (PLA), which subjects consumed 45 minutes prior to RE and in 8h increments for 72h. A randomly counterbalanced, crossover design was utilized so each subject received both treatments. Muscle recovery variables (soreness, muscle function, plasma creatine kinase) were measured 24 and 72 hours following each RE session. Magnitude-based inferences were used to evaluate all outcomes. **RESULTS:** The increase in ascending and descending muscle soreness 24h after the second session of RE (RE₂) was 'likely' less with PLA compared to IBU. Specifically, the reduction in ascending muscle soreness with PLA (RE₁: 43mm to RE₂: 27mm) was absent with IBU (RE₁: 39mm to RE₂: 39mm). A similar response was observed for descending muscle soreness. Furthermore, the impairment in total work performed 72h after RE₂ was 'likely' attenuated with PLA (RE₁: 598J to RE₂: 13.1J), but not IBU (RE₁: 335.1J to RE₂: 343J). Decreases in work performed in the first five reps followed a similar pattern. Finally, the impact of IBU on all muscle strength and CK measurements were 'unclear'. **CONCLUSIONS:** In general, IBU consumption appeared to interfere with the RBE, compared to PLA. Follow-up work is needed to confirm these findings but these preliminary data suggest that prescription-strength dosing of IBU following skeletal muscle trauma may need to be reconsidered.

Chapter 1

Introduction

The earliest documented use of an analgesic substance was willow bark dating back to approximately 300 B.C. (Hersh, 2000). The value of willow bark was originally discovered by Hippocrates and used in Europe throughout the Middle Ages to manage pain associated with childbirth, wounds, ulcers, and inflammation (Hersh, 2000). The analgesic effect of willow bark had been attributed to Salicylic acid, which was later isolated and used to create the first non-steroidal anti-inflammatory drug (NSAID), aspirin. Produced in bulk by Friedrich Bayer and Company, aspirin became available to the public in 1899 (Hersh, 2000). NSAIDs currently account for approximately 30 billion annual drug purchases worldwide (Elnachef, 2008) and are primarily used to treat fever, inflammation, and pain.

NSAIDs deliver their effects by impairing cyclooxygenase (COX) enzyme activity. NSAIDs prevent COX enzymes from converting arachadonic acid to prostaglandins (PG). There are two major COX isoforms expressed in human tissue. COX-1 is found in most tissues and produces PG that control renal function, platelet aggregation, and gastric mucosa maintenance (Green, 2001; Hata, 2004). Whereas the COX-2 isoform is expressed in response to cellular damage and produces PG that regulate inflammatory events. These isoforms are either selectively blocked or simultaneously blocked (non-selective), depending on the NSAID. For instance, Aspirin primarily inhibits COX-1, Celebrex primarily inhibits COX-2, and Ibuprofen (IBU) is non-selective.

NSAID's are extensively used among athletes, especially during acute periods of heavy and/or unaccustomed exercise. Heavy resistance exercise is associated with inflammation, joint stiffness, muscle soreness, and impaired muscle function. Likewise, muscle damage biomarkers such as creatine kinase (CK) (Peake, 2005; Willoughby, 2003; Bruunsgaard, 1997; Newham 1986; Paulsen, 2010), Interleukin-6 (IL-6) (Peake, 2005; Willoughby, 2003; MacIntyre, 2001), and myoglobin (Peake, 2005) are also typically elevated following heavy exercise. Although contemporary research may suggest otherwise (Peterson, 2003; Donnelly, 1990; Krentz, 2008), NSAID use is commonly believed to offset these symptoms.

Not only do COX-1 and COX-2 have regulatory roles in the aforesaid physiology, but they also influence processes involved in cellular adaptations to chronic exercise. Specifically, PGE₂ and PGF_{2a}, manufactured by both COX enzymes, are involved heavily in muscle satellite cell proliferation and differentiation, and protein turnover (Brewer, 2012). PGF_{2a} stimulates protein synthesis whereas PGE₂ stimulates protein degradation, collectively leading to a faster rate of protein turnover (Rodemann, 1982). There is good evidence that NSAIDs inhibit satellite cell proliferation (Mikkelsen, 2009; Mackey 2007), protein synthesis (Trappe, 2002; Burd 2010) and PG synthesis (Markwork, 2014; Trappe, 2001) after acute exercise. Thus, NSAID's taken proximal to heavy exercise to reduce inflammation and pain may interfere with recovery and chronic adaptations. The negative influence of NSAID's on muscle regeneration and adaptation has been shown with animal studies (LaPointe, 2002; Soltow, 2006; Shen, 2006). Few similar studies have been performed in humans. Surprisingly, two separate research groups recently reported that continuous NSAID supplementation either has

minimal effect or actually amplifies muscle growth in response to chronic resistance training (Krentz, 2008, Trappe, 2011). The authors speculated that a parallel pathway may have supercompensated for the COX suppression thereby leading to superior growth (Trappe, 2011).

Little is known about how NSAID use effects non-hypertrophic adaptations, particularly with short-term administration that is similar to what is practiced by athletes. One such adaptation is the repeated bout effect (RBE), where a single session of ‘disruptive’ exercise leads to muscle adaptations that safeguard against similar successive bouts of exercise-induced muscle damage. Repeated exercise is associated with progressively less muscle damage and inflammation (Nosaka, 2001; Eston 1996; Byrnes 2004; Paulsen 2010). For example, evidence of reduced muscle damage (lower serum CK, delayed-onset muscle soreness (DOMS), and myoglobin) has been observed following a second session of downhill running when a previous downhill run (Byrnes, 1985) or isokinetic exercise had been performed (Paulsen, 2010; Eston, 1996). Interestingly, these prophylactic adaptations can last up to 6 months in duration (Nosaka, 2001). The mechanisms responsible for prophylactic adaptations remain unclear but likely include motor unit recruitment strategies, cytoskeletal adaptations and the addition of sarcomeres (McHugh, 2003). These muscle-specific adaptations likely alter protein turnover (Trappe, 2001), satellite cell activity (Mackey, 2007), and inflammation (Lapointe, 2002), all processes that are inhibited by NSAID supplementation. Therefore, it stands to reason that NSAID supplementation may interfere with muscle remodeling involved in the repeated bout effect.

To our knowledge, only one study has examined the influence of NSAIDs on RBE in humans, and the authors reported null findings (Paulsen, 2010). However, subjects consumed Celebrex, a selective COX-2 inhibitor that has minimal effect on COX-1. COX-1 works in conjunction with COX-2 as a partial mediator of exercise-induced protein synthesis (Burd, 2010). Furthermore, COX-2 is likely responsible for post-exercise PG production (Trappe, 2011). As a result of its non-selective nature, IBU has a low-risk profile and seems to have the most impact in the early stages of post-exercise recovery on PG synthesis (Markworth, 2014), PGF_{2a} concentrations (Peterson, 2003; Trappe, 2001) and protein synthesis (Trappe, 2002; Peterson, 2003). It is logical to speculate that IBU may interfere with some of the adaptive characteristics of the RBE, however this thesis has not been examined. Therefore, the primary aim is to investigate the effects of IBU supplementation on the RBE elicited by consecutive sessions of eccentric unilateral knee extensions.

Aims and Hypotheses

Aim 1: To determine if IBU consumption before and after an initial session of eccentric resistance exercise influences skeletal muscle function following a second bout of eccentric resistance exercise, compared to placebo (PLA).

Hypothesis 1: IBU consumption before and after an initial session of eccentric resistance exercise will result in greater peak torque decrements following a second session of eccentric resistance exercise, compared to PLA.

Aim 2: To determine if IBU consumption before and after an initial session of eccentric resistance exercise influences muscle damage (plasma creatine kinase and muscle soreness) following a second session of eccentric resistance exercise, compared to PLA.

Hypothesis 2: IBU consumption before and after an initial session of eccentric resistance exercise will result in more muscle damage following a second session of eccentric resistance exercise, compared to PLA.

Significance

The prevalence of NSAID use combined with preliminary evidence of impaired muscle regeneration and protein turnover during post-exercise recovery points to the value of more research on this topic. Coupling unaccustomed resistance exercise with an anti-inflammatory dose of IBU should create an environment conducive for COX enzyme inhibition of inflammation, satellite cell proliferation and fusion, and protein turnover, thereby maximizing the potential effect that NSAID's may have on the RBE. Given the non-selective nature of IBU, and the partially shared role of both COX-1 and COX-2 isoforms in muscle adaptation, this study will provide insight into the potential for NSAID treatment to impact skeletal muscle physiology. The findings from this study may help fitness professionals, recreational exercisers, and athletes make better informed decisions by weighing short term benefits with possible long term detriments when it comes to NSAID supplementation.

Chapter Two

Methods

Subjects

Eight healthy, active males between the ages of 19 and 32 were recruited from James Madison University. To be included, subjects must not have regularly engaged in lower body resistance exercise (RE) (> 1 session a week within 3 months of study participation). Additionally, subjects must not have consumed any form of NSAID within seven days prior to study participation. Study procedures were approved by the James Madison University Institutional Review Board. Before participation, and after comprehensive verbal and written explanations of the study procedures, all subjects provided written consent. Subject characteristics are presented in Table 1.

Experimental Design

Subjects completed two separate testing phases separated by a washout period of at least 7 days. Each testing phase consisted of two single-leg RE sessions, performed on the same leg, separated by ten days (Figure 1). Each phase was characterized by treatment with either IBU or PLA. A randomly counterbalanced, crossover design was utilized so that each subject received both treatments. For example, subjects ingesting IBU for the first session of RE with Leg A ingested a PLA pill, matched for size and color, for the third session of RE with Leg B, following a 7 day washout period. Prior to any testing, subjects that met the inclusion criteria completed a VO_{2max} test and four RE familiarization trials.

One Repetition Maximum Test (1RM)

Immediately prior to each resistance exercise session, 1RM was tested to determine the workload used in the unilateral eccentric leg extensions. Subjects warmed up on a treadmill at a self-selected pace for 5 minutes. They then performed a warm-up of 10 repetitions of unilateral leg extensions at 20% body weight on a standard leg extension device (Cybex V3 Series, Medway MA, USA). Immediately after, there was 4 minutes of passive recovery followed by two repetitions at 50%-70% of perceived 1 RM. After an additional 4 minutes of passive recovery, subjects attempted their 1 RM and continued to rest in 4-minute segments between attempts until failure.

Resistance Exercise (RE)

The unilateral resistance exercise protocol was adapted from Burd et al. Following 1RM testing, subjects performed 10 sets of 10 repetitions of unilateral eccentric leg extensions with a 60 second rest in between sets. The weight was manually lifted to 180 degrees and lowered to 90 degrees in a 3 second eccentric phase at 120% of the subject's concentric 1-repetition maximum. If the weight was lowered in less than 0.5 seconds, the subject completed that set and the following sets were adjusted in 5 pound increments so they were able to complete the full protocol.

Washout (WO)

A washout (WO) phase of at least 7 days followed the second session of RE for Leg A. During the WO phase, subjects refrained from any ingestion of NSAIDs, and any resistance type exercise. Upon completion of the WO phase, subjects participated in testing phase 2.

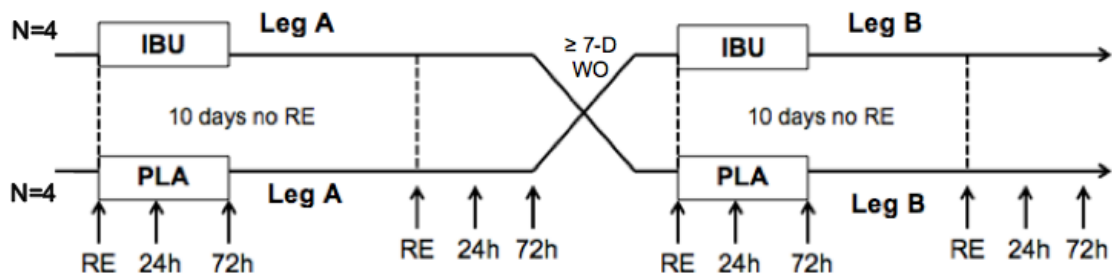


Figure 1. Experimental Design

Non-Steroidal Anti-Inflammatory Drug (NSAID) Treatment

Treatments were administered prior to RE and for 72h following RE. Subjects received either a PLA pill (lactose) or the anti-inflammatory dose of IBU (2400 mg), split into 800mg doses, taken three times a day. The first dose was taken approximately 45 minutes prior to the initial bout of resistance exercise and in subsequent 8h time increments for the next 72 hours. The timing of ingestion immediately after exercise was recorded and standardized between phases. To verify drug compliance, subjects sent a text confirming the ingestion of their assigned treatment.

MEASUREMENTS

Cardiorespiratory Fitness (VO_{2max} Test)

Subjects performed a VO_{2max} test to determine maximal oxygen uptake 7 days prior to the first experimental trial. Subjects rode a computerized cycle ergometer (Velotron, Racermate Inc, Seattle WA) at a self-selected workload estimated as “a comfortable, but not easy pace for a 1-hour ride”. Workload was increased by 25 W every minute until subjects voluntarily requested to stop due to fatigue or are unable to continue at a cadence >50 rpm. Oxygen uptake was assessed during each stage in 30-s

intervals using indirect calorimetry via an automated Moxus Modular Metabolic System (AEI Technologies, Bostrop TX). During the test, heart rate and rate of perceived exertion (RPE) was also recorded.

Skeletal Muscle Function (SMF)

Peak isokinetic concentric muscle force and work performance was assessed following a standardized 5-min warm-up on a treadmill. Skeletal muscle function was assessed six times during each testing phase: Pre-RE 1, RE 1+24h, RE 1+72h, Pre-RE 2, RE 2+24h, RE 2+72h (Figure 1). Subjects were seated and positioned upright in the dynamometer chair with knees bent at a 90-degree angle so the axis of the dynamometer was aligned with the axis of rotation of the knee joint. Adjusting straps were secured across the subject's chest, once the chair settings were properly set, to prevent excess movement associated with each effort. Peak isokinetic concentric torque was assessed by having subjects push as hard as possible against a shin pad connected to an electronic dynamometer that controls the speed of movement through the leg-extension. Subjects performed 2 sets at 30 degrees per second and 2 sets at 120 degrees per second. Each set consisted of 4 repetitions with the first 2 repetitions acting as a warm-up and the last two as maximal efforts. Peak isokinetic eccentric torque was assessed by subjects resisting as hard as possible against the shin pad connected to the electronically controlled dynamometer. The protocol outlined to test peak isokinetic concentric torque was also used to test peak isokinetic eccentric torque. All sets were separated by 60 seconds of rest. Lastly, subjects performed 30 maximum effort leg-extensions at a controlled speed of movement of 120 degrees per second as an assessment of work performance..

Muscle Soreness

Soreness ratings were obtained the day of the damaging trial, and 24h and 72h following each bout of RE. A 100mm visual analog scale, with 0 indicating no muscle soreness and 100 indicating impaired movement due to muscle soreness was used. Subjects completed the scale immediately following ascending, and descending a flight of stairs at normal walking speed.

Venous Blood Draw and Biomarkers

Fasting venous blood samples were obtained from an antecubital vein prior to the start of each RE trial, 24 hours and 72 hours post RE. Upon entering the lab, subjects rested in a blood draw chair for 5 minutes prior to receiving the blood draw. Approximately 10mL of whole blood was obtained at each blood draw, and centrifuged at 3000 rpm for 20 minutes to remove the plasma portion of the blood. Plasma was stored in an -80°C freezer for later analysis. Plasma samples were analyzed for muscle membrane disruption [creatin kinase (CK)]. CK was subsequently analyzed using an automated table-top analyzer (Chemwell-T, Awareness Tech. Inc., Palm City, FL).

DIETARY AND EXERCISE CONTROLS

Prior to the initiation of testing, subjects were provided with portion sizing guides and instructed on how to accurately record dietary intake. Subjects maintained a diet record for 4 days, beginning 24 hours prior to the initial RE trial. Subjects submitted their initial diet records the morning of their first 72h follow-up trial (from the previous 4 days). Subjects refrained from alcohol and caffeine 24 hour and 12 hours prior to the start of RE trials and follow-up visits respectively. Subjects consumed their final “self-

selected” meal no less than 12 hours prior to the start of RE trials and follow-up visits. Subjects were provided with a standardized meal consisting of a 6-oz yogurt, small box of cereal, and orange juice following each RE trial. Subjects consumed the meal within 60 minutes of the RE trial completion and abstained from any further food or beverage intake for the 4 hours following the completion of all RE trials.

Subjects refrained from heavy physical activity 72 hours pre and post RE trial. To ensure compliance, subjects completed a 72h physical activity log prior to each RE trial. Subjects did not engage in any heavy exercise in the previous 24h before each RE trial. In order to avoid any unintended prophylactic adaptations, subjects had not performed any resistance type of exercise within the previous 3 months.

STATISTICAL ANALYSES

Magnitude-based inferences about the data were derived using methods described by Hopkins and colleagues (31). A standardized difference in means (mean difference between treatments divided by the between-subject SD under PLA conditions: SD units) was calculated for each variable whereby observed values equivalent to or exceeding 0.2 SD units was quantified as a substantial treatment effect (i.e. threshold for substantial effect) (31). The 0.2 SD unit threshold was used for all variables.

A published spreadsheet (33) was used to determine the likelihoods of the true treatment effect (of the population) reaching the substantial change threshold (0.2 SD); these were classified as <1% almost certainly no chance, 1-5% = very unlikely, 5-25% = unlikely, 25-75% = possible, 75-95% = likely, 95-99% = very likely, and >99% = almost certain. If the percent chance of the effect reaching the substantial change threshold was

<25% and the effect was clear, it is classified as a 'trivial' effect. If 90% confidence intervals included values that exceeded the substantial change threshold for both a positive and negative effect, effects were classified as 'unclear' (>5% chance of reaching the substantial threshold for both a positive and negative effect). For ease of interpretation data are displayed as raw means \pm SD and/or percent difference between treatments \pm CL (90% confidence limit; to illustrate uncertainty in treatment effects).

Chapter Three

Manuscript

Abstract

PURPOSE: This proof of concept study was designed to assess the influence of prescription-strength Ibuprofen (IBU) on the repeated bout effect (RBE) of heavy exercise. **METHODS:** Eight males (23 ± 4 yrs) with no recent history of lower-body resistance training completed two separate testing phases separated by a seven-day washout period. Each phase consisted of two sessions of single-legged resistance exercise (RE), performed on the same leg, separated by ten days. One RE trial included 10 sets of 10 repetitions of maximal unilateral eccentric leg extensions at 120% 1RM. Each phase was characterized by a distinct treatment of either 800mg IBU or placebo (PLA), which subjects consumed 45 minutes prior to RE and in 8h increments for 72h. A randomly counterbalanced, crossover design was utilized so each subject received both treatments. Muscle recovery variables (soreness, muscle function, plasma creatine kinase) were measured 24 and 72 hours following each RE session. Magnitude-based inferences were used to evaluate all outcomes. **RESULTS:** The increase in ascending and descending muscle soreness 24h after the second session of RE (RE₂) was 'likely' less with PLA compared to IBU. Specifically, the reduction in ascending muscle soreness with PLA (RE₁: 43mm to RE₂: 27mm) was absent with IBU (RE₁: 39mm to RE₂: 39mm). A similar response was observed for descending muscle soreness. Furthermore, the impairment in total work performed 72h after RE₂ was 'likely' attenuated with PLA (RE₁: 598J to RE₂: 13J), but not IBU (RE₁: 335J to RE₂: 343J). Decreases in work performed in the first five reps followed a similar pattern. Finally, the impact of IBU on all muscle strength and CK measurements were 'unclear'. **CONCLUSIONS:** In general, IBU consumption appeared to interfere with the RBE, compared to PLA. Follow-up work is needed to confirm these findings but these preliminary data suggest that prescription-strength dosing of IBU following skeletal muscle trauma may need to be reconsidered.

Introduction

NSAIDs (Non-Steroidal Anti-inflammatory Drugs) currently account for approximately 30 billion annual drug purchases worldwide (Elnachef, 2008) and are primarily used to treat fever, inflammation, and pain. NSAIDs deliver their effects by impairing cyclooxygenase (COX) enzyme activity. NSAIDs prevent COX enzymes from converting arachadonic acid to prostaglandins (PG). There are two major COX isoforms expressed in human tissue. COX-1 is found in most tissues and produces PG that control renal function, platelet aggregation, and gastric mucosa maintenance (Green, 2001; Hata, 2004). The COX-2 isoform is expressed in response to cellular damage and produces PG that regulate inflammatory events. These isoforms are either selectively blocked or simultaneously blocked (non-selective), depending on the NSAID.

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Not only do COX-1 and COX-2 have regulatory roles in the aforestated physiology, but they also influence processes involved in cellular adaptations to chronic exercise. Specifically, PGE₂ and PGF_{2a}, manufactured by both COX enzymes, are involved heavily in muscle satellite cell proliferation and differentiation, and protein

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To our knowledge, only one study has examined the influence of NSAIDs on RBE in humans, and the authors reported null findings (Paulsen, 2010). However, subjects consumed Celebrex, a selective COX-2 inhibitor. COX-1 works in conjunction with COX-2 as a partial mediator of exercise-induced protein synthesis (Burd, 2010). Furthermore, COX-2 is likely responsible for post-exercise prostaglandin production (Trappe, 2012). IBU has a low-risk profile and seems to impact several key processes during the early stages of post-exercise recovery including PG synthesis (Markworth, 2014), PGF_{2a} concentrations (Peterson, 2003; Trappe, 2001) and protein synthesis (Trappe, 2002; Peterson, 2003). Therefore, the primary aim of this study is to investigate the effects of IBU supplementation on the RBE elicited by consecutive sessions of eccentric unilateral knee extensions.

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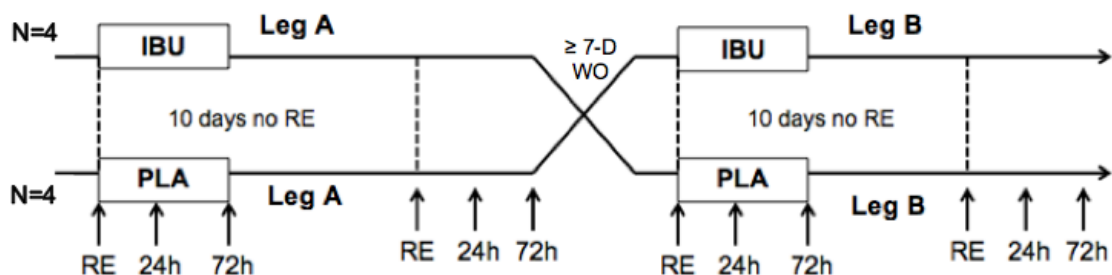


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DIETARY AND EXERCISE CONTROLS

Prior to the initiation of testing, subjects were provided with portion sizing guides and instructed on how to accurately record dietary intake. Subjects maintained a diet record for 4 days, beginning 24 hours prior to the initial RE trial. Subjects submitted their initial diet records the morning of their first 72h follow-up trial (from the previous 4 days). Subjects refrained from alcohol and caffeine 24 hour and 12 hours prior to the start

of RE trials and follow-up visits respectively. Subjects consumed their final “self-selected” meal no less than 12 hours prior to the start of RE trials and follow-up visits. Subjects were provided with a standardized meal consisting of a 6-oz yogurt, small box of cereal, and orange juice following each RE trial. Subjects consumed the meal within 60 minutes of the RE trial completion and abstained from any further food or beverage intake for the 4 hours following the completion of all RE trials.

Subjects refrained from heavy physical activity 72 hours pre and post RE trial. To ensure compliance, subjects completed a 72h physical activity log prior to each RE trial. Subjects did not engage in any heavy exercise in the previous 24h before each RE trial. In order to avoid any unintended prophylactic adaptations, subjects had not performed any resistance type of exercise within the previous 3 months.

STATISTICAL ANALYSES

Magnitude-based inferences about the data were derived using methods described by Hopkins and colleagues (31). A standardized difference in means (mean difference between treatments divided by the between-subject SD under PLA conditions: SD units) was calculated for each variable whereby observed values equivalent to or exceeding 0.2 SD units was quantified as a substantial treatment effect (i.e. threshold for substantial effect) (31). The 0.2 SD unit threshold was used for all variables.

A published spreadsheet (33) was used to determine the likelihoods of the true treatment effect (of the population) reaching the substantial change threshold (0.2 SD); these were classified as <1% almost certainly no chance, 1-5% = very unlikely, 5-25% = unlikely, 25-75% = possible, 75-95% = likely, 95-99% = very likely, and >99% = almost

certain. If the percent chance of the effect reaching the substantial change threshold was <25% and the effect was clear, it is classified as a 'trivial' effect. If 90% confidence intervals included values that exceeded the substantial change threshold for both a positive and negative effect, effects were classified as 'unclear' (>5% chance of reaching the substantial threshold for both a positive and negative effect). For ease of interpretation data are displayed as raw means \pm SD and/or percent difference between treatments \pm CL (90% confidence limit; to illustrate uncertainty in treatment effects).

RESULTS

Muscle Soreness

Soreness values following ascension and descension of one flight of stairs 24h after RE are displayed in Figure 1. Data in parenthesis represents the difference between the two repeated RE trials for each treatment. RE₁ indicates the 1st initial bout of damaging exercise while RE₂ indicates the 2nd subsequent damaging bout of exercise for one leg.

Ascending stairs

There was a '*likely*' treatment effect on the RBE for ascending soreness at the 24-hr mark. With PLA, the increase in ascending soreness following RE₁ (43mm) was '*very likely*' greater than the increase in soreness 24 hrs following RE₂ (27mm). Conversely, there were no clear changes in soreness 24 hrs after RE₁ (39mm) versus RE₂ (39mm) with IBU.

There were no clear treatment differences in soreness at the 72-hr time point. Ascending soreness following RE₁ (40mm) was '*likely*' greater than RE₂ (15mm) with PLA. The IBU treatment showed a similar response with soreness '*likely*' greater after RE₁ (30mm) compared to RE₂ (21mm).

Descending stairs

There was a '*likely*' treatment effect on the RBE for descending soreness at the 24-hr mark. With PLA, the increase in descending soreness following RE₁ (55mm) was '*very likely*' greater than the increase in soreness 24 hrs following RE₂ (31mm).

Conversely, there were no clear changes in soreness 24 hrs after RE₁ (48mm) versus RE₂ (42mm) with IBU.

There were no clear treatment differences in soreness at the 72-hr time point. Descending soreness following RE₁ (47mm) was '*very likely*' greater than RE₂ (20mm) with PLA. The IBU treatment showed a similar response with soreness '*likely*' greater after RE₁ (35mm) compared to RE₂ (21mm).

Whole Muscle Function

Peak muscle torque values are displayed in Table 2.

Concentric Isokinetic Peak Torque

There were no clear treatment differences on the RBE at both 24 hrs and 72 hrs for isokinetic peak torque at $30 \text{ deg}\cdot\text{sec}^{-1}$ and $120 \text{ deg}\cdot\text{sec}^{-1}$. Decrements in torque at $30 \text{ deg}\cdot\text{sec}^{-1}$ 24 hrs after RE₂ (33 Nm) were '*possibly*' lower compared to RE₁ (49 Nm) with PLA. However, it was not clear if decreases in torque were less after RE₂ (37 Nm) compared to RE₁ (45 Nm) with IBU. Likewise, declines in torque 72 hrs post RE₂ (11 Nm) were '*likely*' smaller in comparison to RE₁ (41 Nm) with PLA. Similarly, recovery after RE₂ (23 Nm) compared to RE₁ (22 Nm) was not clear with IBU.

It was not clear if decreases in torque at $120 \text{ deg}\cdot\text{sec}^{-1}$ 24 hrs after RE₁ (33 Nm) were regained after RE₂ (30 Nm) with PLA. Contrarily, torque decrements '*likely*' diminished after RE₂ (23 Nm) compared to RE₁ (38 Nm) with IBU. Declines in peak torque 72h following RE₁ (34 Nm) were '*likely*' restored after RE₂ (8 Nm) with PLA. However, it was not clear if decrements in torque recovered after RE₂ (12 Nm) compared to RE₁ (17 Nm) with IBU.

Eccentric Isokinetic Peak Torque

There were no clear treatments effects on the RBE at both 24 hrs and 72 hrs for isokinetic peak torque at $30 \text{ deg}\cdot\text{sec}^{-1}$ and $120 \text{ deg}\cdot\text{sec}^{-1}$. Decrements in torque at $30 \text{ deg}\cdot\text{sec}^{-1}$ 24 hrs following RE₁ (44 Nm) were ‘possibly’ lower after RE₂ (28 Nm) with PLA. It was not clear if the same was seen after RE₂ (45 Nm) compared to RE₁ (62 Nm) with IBU. Likewise, declines in torque 72 hrs after RE₂ (-5 Nm) were ‘likely’ smaller in comparison to RE₁ (25 Nm) with PLA. It was not clear if similar recovery after RE₂ (10 Nm) compared to RE₁ (17 Nm) was occurred with IBU.

Decreases in torque at $120 \text{ deg}\cdot\text{sec}^{-1}$ 24 hrs after RE₁ (60 Nm) were ‘likely’ regained after RE₂ (22 Nm) with PLA. Comparably, torque decrements ‘likely’ diminished after RE₂ (38 Nm) compared to RE₁ (67 Nm) with IBU. It was not clear if restoration of peak torque 72 hrs following RE₁ (34 Nm) happened after RE₂ (-10 Nm) with PLA. Declines in torque were ‘possibly’ reduced after RE₂ (3 Nm) compared to RE₁ (17 Nm).

Whole Muscle Performance

Total work values are displayed in Figure 2 and work done in the first 5 reps is displayed in Figure 3.

Work

There were ‘likely’ treatment effects at both 24 hrs and 72 hrs for total work done and work performed within the first five reps (FFR). The impairment in total work 24 hrs following RE₂ (365 J) was ‘very likely’ less compared to RE₁ (1,005 J) with PLA. With IBU, the decrement was ‘possibly’ less after RE₂ (590 J) as opposed to RE₁ (865 J). This

same pattern was observed at 72 hrs as decreases in work performed '*likely*' recovered from RE₁ (598 J) to RE₂ (13 J) with PLA. It was not clear if differences existed between RE₁ (335 J) and RE₂ (343 J) with IBU.

Furthermore, the decrease in FFR 24 hrs following RE₁ (307 J) '*very likely*' improved after RE₂ (116 J) with PLA. However, it was not clear if FFR was less impaired after RE₂ (178 J) compared to RE₁ (255 J) with IBU. In accordance, the reduction in FFR 72 hrs following RE₁ (206 J) '*likely*' improved following RE₂ (9 J) with PLA. There was no clear improvement from RE₁ (84 J) to RE₂ (73) with IBU.

Creatine Kinase Levels

CK levels are displayed in Table 3.

Plasma Creatine Kinase (CK)

There were no clear treatment effects on the RBE at both 24 hr and 72 hr time points. The plasma CK response 24 hr following RE₂ (90 U/L) was '*likely*' diminished compared to RE₁ (144 U/L) with PLA. Similarly, it was '*likely*' that CK was attenuated after RE₂ (68 U/L) compared to RE₁ (145 U/L) with IBU. CK response 72h after RE₁ (1590 U/L) was '*very likely*' larger compared to RE₂ (11 U/L) with PLA. However, with IBU this was not clear as RE₁ (686 U/L) was similar to RE₂ (-1 U/L).

DISCUSSION

The primary goal of this proof-of-concept investigation was to determine if short-term IBU consumption interferes with well-documented skeletal muscle prophylactic adaptations that occur after an initial bout of heavy unaccustomed exercise, otherwise known as the RBE. We quantified the magnitude of the RBE by assessing muscle function and perceived soreness after two identical eccentric exercise sessions (unilateral leg extension) separated by 10 days. Contralateral legs were exposed to this design, one with acute IBU and the other with PLA. The main finding was that IBU appeared to impair the RBE, compared to PLA.

It is well established that after an initial bout of damaging exercise, any subsequent session of similarly heavy exercise is ensued by comparatively less muscle soreness (Eston, 1996; Byrnes, 1985) and attenuated impairments in muscle function (Eston, 1996). A RBE clearly occurred in the present study at both 24 hrs and 72 hrs following damaging exercise in virtually all of the markers during the PLA treatment (soreness, 30 deg/sec concentric and eccentric peak torque at 72h, 120 deg/sec concentric peak torque at 72h, 120 deg/sec eccentric peak torque at 24h, total work, work performed in the first 5 reps and CK). Evidence of a RBE with IBU was less consistent (soreness at 72h, 120 deg/sec concentric and eccentric peak torque at 24h, and CK at 24h). These separate adaptations lead to clear treatment differences in the RBE between IBU and PLA, altogether suggesting that IBU inhibits prophylactic adaptations. There is only one similar study available for comparison and it contradicts the current data (Paulsen, 2010). However, Paulsen used a smaller drug dose (400mg) and a different class of NSAID (Celebrex – COX-2 Inhibitor). We administered IBU to target both COX-1 and COX-2

pathways, as COX-1 is thought to be involved in cellular pathways necessary for skeletal muscle remodeling such as protein synthesis and satellite cell proliferation. Specifically, COX-1 is believed to be involved in the increase in protein synthesis following exercise (Burd, 2010) and PG production necessary for early signaling post-exercise recovery (Markworth, 2014). Indeed, IBU has been shown to suppress protein synthesis (Trappe, 2002) and PGF_{2a} production (Trappe 2001) following exercise, while COX-2 inhibition has not (Burd, 2010). Similarly, satellite cell proliferation has also occurred through the use of nonselective COX inhibitors similar to IBU (Mackey, 2007; Mikkelsen, 2010) but not with COX-2 inhibitors (Paulsen, 2010). Another possible explanation for the discrepant findings is that Paulson administered 400mg of Celebrex twice a day compared to the 800 mg IBU dose taken thrice a day in the present study. The dosage used by Paulsen is not considered anti-inflammatory (Abramson, 1989) and may not be a large enough stimulus to cause any disruption in muscle regeneration and function.

In humans, long-term effects of using non-selective COX inhibitors, Naproxen sodium and IBU, have been documented (Krentz, 2008; Brewer, 2015). Although NSAID supplementation had negative effects on early adaptations to resistance exercise in the more recent study, these impacts diminished over the course of the 6-week resistance training program and had no repercussions on morphological adaptations (Brewer, 2015). Comparably, IBU did not influence muscle hypertrophy or strength following 6 weeks of resistance training in the earlier investigation (Krentz, 2008). Though it is difficult to extrapolate the current data, it is possible that the negative impact of IBU on the RBE in the first 10 days observed here may dissipate over time. Though the differences in exercise stimulus and amount of IBU taken may affect skeletal muscle remodeling for an

extended duration. In rebuttal, one animal study communicates how non-selective COX inhibition may have negative long-term consequences on skeletal muscle. Concerning findings were revealed when rabbits were administered Flurbiprofen (non-selective COX inhibitor) (Mishra, 2006). Those treated with the NSAID enhanced muscle function more so than the controls at days 3 and 7 after one damaging bout of exercise. However, 28 days later torque deficits increased and force production decreased in the Flurbiprofen group (Mishra, 2006). It is worthy to note that while this study did not examine the RBE, the associated long-term impairment hints at the possible consequences of using non-steroidal anti-inflammatory drugs.

The current research design also allowed us to provide insight into the prophylactic efficacy of IBU following acute exercise. The aforementioned dosage of 2400mg of IBU has only been used one other time to our knowledge (Pizza, 1998). Findings indicated prophylactic IBU consumption did not influence peak torque and isometric strength after performing one bout of eccentric arm curls (Pizza, 1998). Conversely, NSAID use has assisted with an increase in muscle function acutely after heavy exercise (Hasson, 1993; Mishra, 2006). This was not the case within the present study as it was 'unclear' if muscle soreness, CK, peak torque and work performed benefited from IBU consumption after the initial bout. These findings are in agreement that IBU consumption does not influence muscle soreness (Bourgeois, 1999; Krentz, 2008; Trappe, 2001) or muscle damage (Bourgeois, 1999).

The subjects in the current study had a wide range of body masses; 69 to 105 kg, potentially adding variability to the outcomes. Although it has yet to be thoroughly examined, it is possible that 2400mg of IBU administered to individuals of a larger body

mass may not elicit the same response as subjects with less body mass. However, a previous investigation with individuals of similar mass to the current study found that 1200mg of IBU elevated serum IBU concentrations and attenuated PG synthesis to a similar extent across subjects (Markworth, 2014). Therefore, the current dose was likely enough to suppress inflammatory processes in all subjects. Furthermore, the correlation between body mass and the affect of IBU on the RBE was 0.045, suggesting that body mass had virtually no influence on the results.

In general, the data indicate that IBU consumption blunted the RBE. This was most obvious for muscle soreness and leg extension work. To our knowledge, this is the first evidence that acute IBU consumption proximal to initial damaging exercise hinders tolerance to the subsequent bout of heavy exercise. We recommend thoughtful consideration before consuming IBU as a recovery aid, as it may negatively influence adaptations necessary to protect the muscle from subsequent bouts of damaging exercise.

Table 1. Descriptive Characteristics

	Height (cm)	Mass (kg)	Age (yrs)	VO _{2max} (ml/kg/min)
N = 8	179.6 ± 7.0	82.1 ± 12.3	22.8 ± 4.1	45.3 ± 7.0

Data are displayed as mean ± SD.

Table 2. Raw peak isokinetic torque values (Nm)

Variable	Treatment	Speed (deg·sec ⁻¹).	RE ₁			RE ₂		
			Pre	24h	72h	Pre	24h	72h
Concentric	IBU	30	221.0 ± 57.9	175.6 ± 62.7	199.3 ± 63.9	223.1 ± 58.3	186.0 ± 61.2	199.6 ± 52.5
		120	197.3 ± 32.2	159.7 ± 35.2	180.8 ± 43.2	195.8 ± 29.1	172.9 * ± 33.5	184.1 ± 33.2
	PLA	30	218.0 ± 67.7	169.3 ± 56.7	176.8 ± 78.7	215.5 ± 59.9	183.0 * ± 48.8	205 ± 49.6
		120	199.6 ± 34.4	167.1 ± 33.2	165.2 ± 52.1	192.2 ± 29.5	162.3 ± 24.0	184.3* ± 23.3
Eccentric	IBU	30	278.7 ± 81.6	216.5 ± 71.3	261.8 ± 83.8	270.5 ± 87.0	226.0 ± 70.7	260.9 ± 88.6
		120	289.0 ± 65.5	221.9 ± 78.1	271.8 ± 85.1	271.2 ± 63.9	233.0 * ± 72.4	267.9 ± 72.4
	PLA	30	247.3 ± 58.7	203.8 ± 64.2	222.2 ± 70.7	250.6 ± 69.3	222.8 ± 78.0	255.9* ± 79.5
		120	270.8 ± 52.0	210.6 ± 55.9	237.3 ± 82.8	260.8 ± 77.0	239.3 * ± 63.9	270.4 ± 69.8

Data are displayed as mean ± SD * RBE 'likely'

Table 3. CK values (U/L) Pre, 24h and 72h post RE.

Treatment		Pre	24h	72h
IBU	RE ₁	114.8 ± 31.2	259.3 ± 147.8	800.7 ± 1360.3
	RE ₂	124.7 ± 35.4	192.6 ± 59.1	118.5 ± 28.5
PLA	RE ₁	127.4 ± 66.8	271.8 ± 111.7	1515.8 ± 1992.1
	RE ₂	143.0 ± 46.6	233.3 ± 84.0	143.5 ± 56.4

Data are displayed as mean ± SD *RBE 'likely'

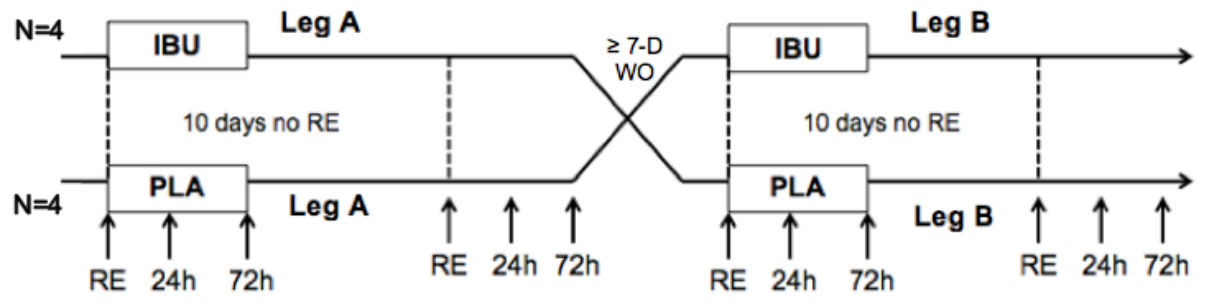
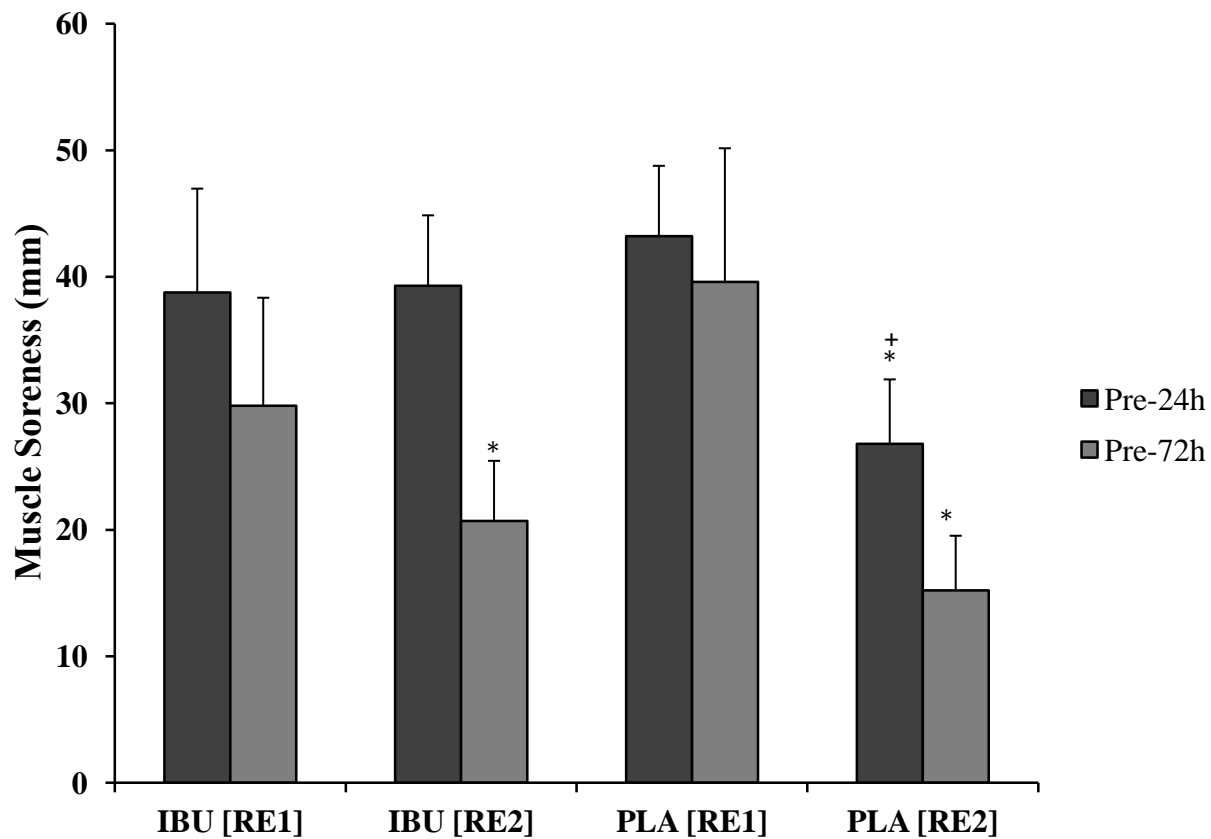


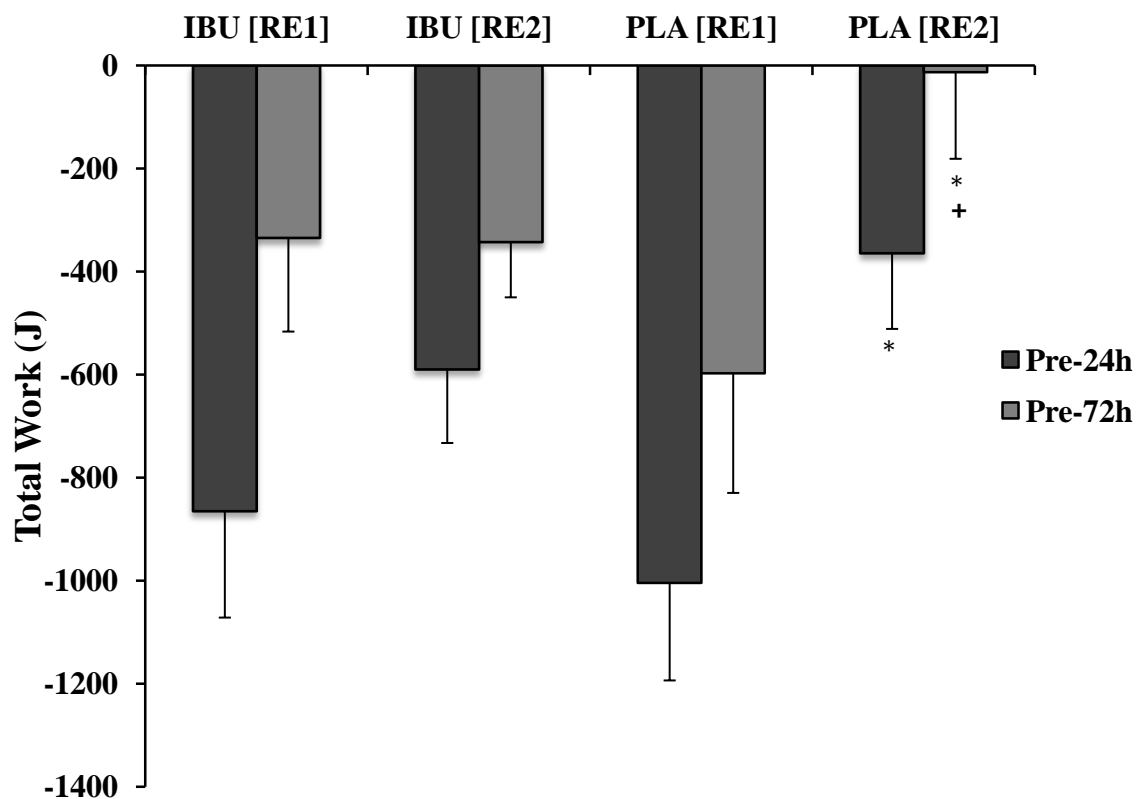
Figure 1. Experimental Design

Figure 2. Difference in perceived ascending muscle soreness from pre to 24 hrs and pre to 72h following RE.



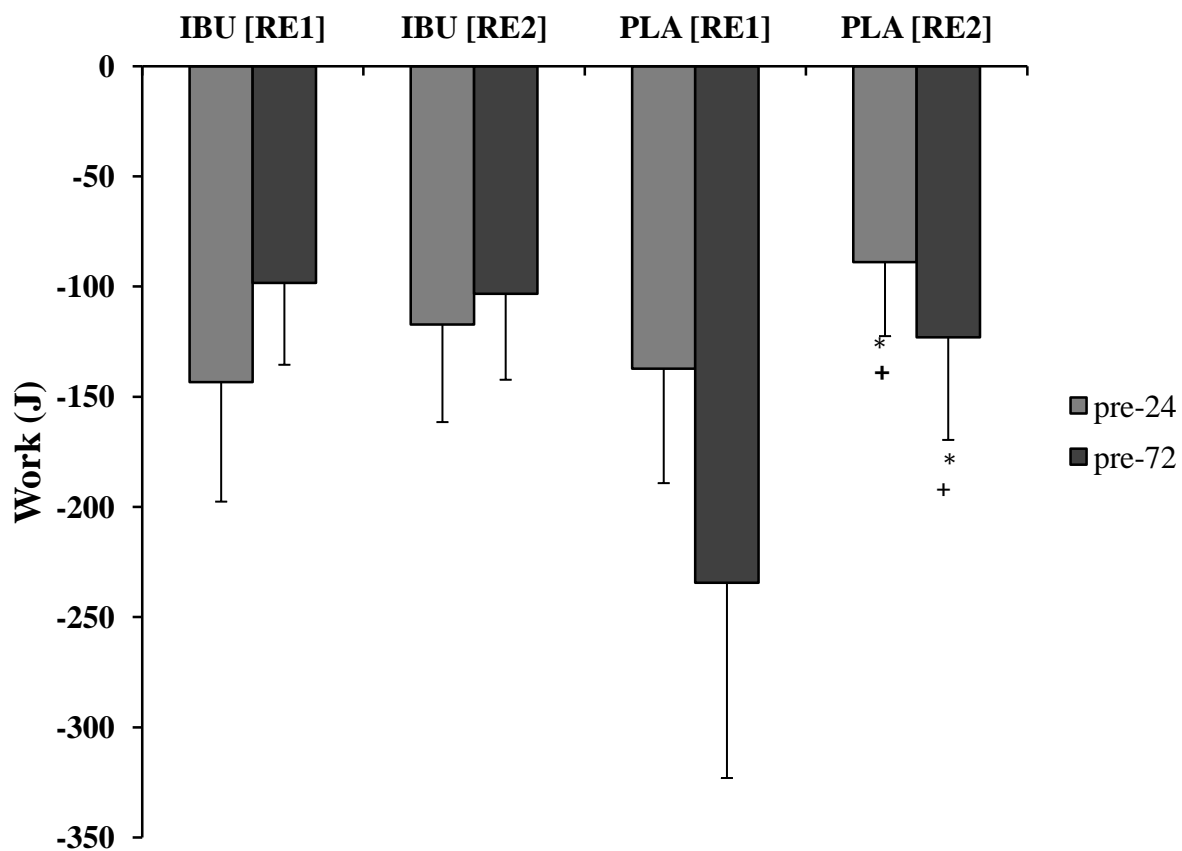
[RE2] values represent muscle soreness assessed 10 days after the first IBU or PLA trial [RE1]. Data are displayed as mean \pm SE.

Figure 3. Difference in total work performed from pre to 24h and pre to 72h.



[RE2] values represent work performed 10 days after the first IBU or PLA trial [RE1]. Data are displayed as mean \pm SE. *RBE 'likely'
 + Difference in treatment 'likely'

Figure 4. Difference in work performed during the first five reps from pre to 24h and to 72h.



[RE2] values represent work performed 10 days after the first IBU or PLA trial [RE1]. Data are means \pm SE.

*RBE 'likely'

+ Difference in treatment 'likely'

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Appendix A

Maximum VO₂ Data Table

Bike

Date _____

Subject _____

Age _____

Height (cm) _____

Gender _____

Body Weight (kg) _____

Resting HR _____

Seat Height: _____

Handlebar Height: _____

Seat Fore/aft: _____

Handlebar fore/aft: _____

Have subject rest for 5 minutes for resting HR

Nosepiece

Subject will determine starting workload; Increase workload 25 W every 1 min

Time (min)	Work-Watts	HR (bpm)	VO ₂ (L/min)	VO ₂ (ml/kg/min)	RPE
3 min warm-up	75				
Exercise min 1					
Min 2					
Min 3					
Min 4					
Min 5					
Min 6					
Min 7					
Min 8					
Min 9					
Min 10					
Min 11					
Min 12					
Min 13					
Min 14					
Min 15					
Min 16					
Min 17					
Min 18					
Min 19					
Min 20					

Appendix B

PAIN Study
Familiarization Trial

Subject #: _____ Date: _____

Trial: FAM1 FAM2 Leg Involved: Left Right

Explain the protocol in detail to ensure the subject knows what is expected.

5 minute warm up on treadmill at 3.0 mph

BIODEX

Chair position: _____

Seatback position: _____

Machine position: _____

Seat height: _____

Arm attachment position: _____

Speed	Concentric PeakT	Eccentric PeakT
30	_____	_____
30	_____	_____
120	_____	_____
120	_____	_____

Fatigability

Work 1st third

Work Last third

Fatigue Index: _____

Appendix C**Muscle Soreness Questionnaire**

Subject # _____

Date: _____

Trial: A B C D

Circle: Pre 24h 72h

Leg Involved: Right Left

Muscle Soreness

➔ Please place a mark on the line below corresponding to your level of muscle soreness

0 millimeters (left) = complete absence of muscular soreness

100 millimeters (right) = extremely sore with noticeable pain and stiffness at all times

Pressure while seated:

0 mm _____ 100 mm

Ascending 1 flight of stairs:

0 mm _____ 100 mm

Descending 1 flight:

0 mm _____ 100 m

Notes:

Appendix D**PAIN Study**
Experimental Trial

Subject #: _____

Date: _____

Trial: A B C D

Circle: Pre 24h 72h

Leg Involved: Left Right

Explain the protocol in detail to ensure the subject knows what is expected.

5 minute warm up on treadmill at 3.0 mph

BIODEX

Chair position: _____

Seatback position: _____

Machine position: _____

Seat height: _____

Arm attachment position: _____

Speed	Concentric PeakT	Eccentric PeakT
30	_____	_____
30	_____	_____
120	_____	_____
120	_____	_____

Fatigability**Work 1st third****Work Last third**

Fatigue Index: _____

Appendix E**PAIN Study**
Experimental Trial

Subject Number: _____

Date: _____

Trial: A B C D

1 repetition maximum protocol:

10 warm up reps at 20% body weight

4 minutes of passive recovery

2 reps at 50-70% of their perceived 1RM

4 min of passive recovery

1RM attempt

4 min of passive recovery

Repeat until failure

20% body weight: _____ lbs.**50-70% perceived 1RM:** _____ lbs.

First 1RM attempt: _____ lbs.

Second 1RM attempt: _____ lbs.

Third 1RM attempt: _____ lbs.

Fourth 1RM attempt: _____ lbs.

Fifth 1RM attempt: _____ lbs.

Sixth 1RM attempt: _____ lbs.

1RM: _____ lbs.**120% of 1RM:** _____ lbs.**Damaging protocol:**

10 sets of 10 repetitions at 120% 1RM

1. Set # when weight was lowered: _____
Weight lowered to: _____ lbs.2. Set # when weight was lowered: _____
Weight lowered to: _____ lbs.3. Set # when weight was lowered: _____
Weight lowered to: _____ lbs.

Appendix F

**James Madison University
Human Research Review Request**

FOR IRB USE ONLY:	
Exempt:	<input type="checkbox"/> Protocol Number: _____ 1st Review: _____ Reviewer: _____
Expedite:	<input type="checkbox"/> IRB: <u>15-0445</u> 2nd Review: <u>X</u> Reviewer: _____
Full Board:	<input checked="" type="checkbox"/> Received: <u>02/16/15</u> 3rd Review: _____
<hr/>	
Project Title:	Does Ibuprofen Interfere With Prophylactic Adaptations to Heavy Exercise?
Project Dates: (Not to exceed 1 year minus 1 day)	From: <u>2/15/15</u> To: <u>2/14/16</u> MM/DD/YY MM/DD/YY
<hr/>	
Minimum # of Participants:	<u>8</u>
Maximum # of Participants:	<u>12</u>
<hr/>	
External Funding:	Yes: <input type="checkbox"/> No: <input checked="" type="checkbox"/> If yes, Sponsor: _____
	Internal Funding: Yes: <input checked="" type="checkbox"/> No: <input type="checkbox"/>
	Will monetary incentives be offered with funding? Yes: <input checked="" type="checkbox"/> No: <input type="checkbox"/>
	If yes: How much per recipient? <u>Three drawings for \$125 each</u> In what form? <u>Check</u>
Must follow JMU Financial Policy:	http://www.jmu.edu/financemanual/procedures/4205.shtml#.391Incentives
<hr/>	

Responsible Researcher(s) :	<u>Nicholas D. Luden, Ph.D.</u>	
E-mail Address:	<u>Jessica G. Ehrbar</u>	
Telephone:	<u>540-568-4069</u>	
Department:	<u>Kinesiology</u>	
Address (MSC):	<u>2302</u>	
Please Select:	<input checked="" type="checkbox"/> Faculty	<input type="checkbox"/> Undergraduate Student
(if Applicable):	<input type="checkbox"/> Administrator/Staff Member	<input type="checkbox"/> Graduate Student
Research Advisor:	_____	
E-mail Address:	_____	
Telephone:	_____	
Department:	_____	
Address (MSC):	_____	

Investigator: Please respond to the questions below. The IRB will utilize your responses to evaluate your protocol submission.

- YES** **NO** Does the James Madison University Institutional Review Board define the project as *research*?

The James Madison University IRB defines "research" as a "systematic investigation designed to develop or contribute to *generalizable knowledge*." All research involving human participants conducted by James Madison University faculty and staff and students is subject to IRB review.
- YES** **NO** Are the human participants in your study *living* individuals?

"Individuals whose physiologic or behavioral characteristics and responses are the object of study in a research project. Under the federal regulations, human subjects are defined as: living individual(s) about whom an investigator conducting research obtains:

(1) data through intervention or interaction with the individual; or (2) identifiable private information."
- YES** **NO** Will you obtain data through *intervention* or *interaction* with these individuals?

“Intervention” includes both physical procedures by which data are gathered (*e.g.*, measurement of heart rate or venipuncture) and manipulations of the participant or the participant's environment that are performed for research purposes. “Interaction” includes communication or interpersonal contact between the investigator and participant (*e.g.*, surveying or interviewing).

4. **YES** **NO** Will you obtain *identifiable private information* about these individuals?

"Private information" includes information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place, or information provided for specific purposes which the individual can reasonably expect will not be made public (*e.g.*, a medical record or student record). "Identifiable" means that the identity of the participant may be ascertained by the investigator or associated with the information (*e.g.*, by name, code number, pattern of answers, etc.).

-
5. **YES** **NO** Does the study present *more than minimal risk* to the participants?

"Minimal risk" means that the risks of harm or discomfort anticipated in the proposed research are not greater, considering probability and magnitude, than those ordinarily encountered in daily life or during performance of routine physical or psychological examinations or tests. Note that the concept of risk goes beyond physical risk and includes psychological, emotional, or behavioral risk as well as risks to employability, economic well being, social standing, and risks of civil and criminal liability.

CERTIFICATIONS:

For James Madison University to obtain a Federal Wide Assurance (FWA) with the Office of Human Research Protection (OHRP), U.S. Department of Health & Human Services, **all** research staff working with human participants must sign this form and receive training in ethical guidelines and regulations. "Research staff" is defined as persons who have direct and substantive involvement in proposing, performing, reviewing, or reporting research and includes students fulfilling these roles as well as their faculty advisors. The Office of Research Integrity maintains a roster of all researchers who have completed training within the past three years.

Test module at ORI website

<http://www.jmu.edu/resandearchintegrity/irb/irbtraining.shtml>

Name of Researcher(s) and Research Advisor	Training Completion Date
Nicholas D. Luden	
Jessica G. Ehrbar	
Dr. Kent Diduch, M.D.	

For additional training interests, or to access a Spanish version, visit the National Institutes of Health Protecting Human Research Participants (PHRP) Course at: <http://phrp.nihtraining.com/users/login.php>.

By signing below, the Responsible Researcher(s), and the Faculty Advisor (if applicable), certifies that he/she is familiar with the ethical guidelines and regulations regarding the protection of human research participants from research risks. In addition, he/she agrees to abide by all sponsor and university policies and procedures in conducting the research. He/she further certifies that he/she has completed training regarding human participant research ethics within the last three years.

Principal Investigator Signature

Date

Principal Investigator Signature

Date

Principal Investigator Signature

Date

Faculty Advisor Signature

Date

Submit an electronic version (in a Word document) of your **ENTIRE** protocol to researchintegrity@jmu.edu.

Provide a **SIGNED** hard copy of the Research Review Request Form to:
Office of Research Integrity, MSC 5738, 601 University Boulevard, Blue Ridge Hall, Third Floor, Room # 342

Purpose and Objectives

Rationale

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used after performing unaccustomed exercise, presumably in an attempt to manage the resulting inflammation and soreness/stiffness. Mechanistically, NSAIDs elicit their effect by interfering with the COX enzyme (COX-1 and/or COX-2) pathway to prevent the conversion of arachadonic acid to prostaglandins (3). While COX inhibition may transiently mask the acute detrimental consequences of heavy exercise (soreness and inflammation) (4,7), manipulation of this pathway also influences cellular processes needed for desirable adaptations to repeated exercise. Specifically, various NSAIDs (both COX-2 and COX-1/COX-2 inhibitors) can blunt post-exercise protein synthesis and satellite cell proliferation (5,9). Therefore, it is logical to speculate that NSAID supplementation may interfere with select cellular adaptations to heavy exercise.

The prophylactic effect of exercise is a hallmark adaptation that commences following the first session of naïve exercise so that subsequent bouts of identical exercise elicit progressively less damage and functional impairment. While one recent study reported that short-term COX-2 inhibition (Celebrex) does not interfere with prophylactic adaptations (7), there is good reason to believe that simultaneous COX-1 and COX-2 inhibition (Ibuprofen) is more potent and therefore may be more likely to negatively impact prophylactic adaptations. Moreover, previous research on the topic used the largest over the counter dose of Ibuprofen (1200 mg/day) despite the fact that it is well known that prescription strength Ibuprofen doses (2400 mg/day) not only relieves pain but also influences inflammation, which has an important role in tissue remodeling. Thus, the aim of the proposed study is to test the hypothesis that acute prescription strength Ibuprofen treatment will impair prophylactic adaptations, thereby leading to greater muscle damage and impaired recovery following a subsequent session of heavy resistance exercise (following drug washout), compared to a placebo treatment.

Note: All potential subjects will be seen by Dr. Diduch at the James Madison University student health center where they will complete the health history questionnaire, screening questionnaire (attachments B & E) and be health screened. Low risk subjects (described below) will receive a prescription for placebo (800 mg 3 x day for 72 hours – 9 doses), and Ibuprofen (800 mg 3 x day for 72 hours) to be filled at the James Madison University student health center pharmacy. JMU pharmacist Ron French has agreed to prepare bottles of placebo and Ibuprofen and to dispense the bottles to our research subjects. Copies of screening materials will be kept on file at the health center and copies will be obtained for our records at the Human Performance Laboratory.

Significance

These findings may be used to help fitness professionals, recreational exercisers, and athletes make better-informed decisions regarding NSAID supplementation, as they weigh short-term benefits against possible long-term consequences.

Procedures

Source and Number of Subjects

8-12 recreationally active male adults will be recruited from James Madison University and the surrounding area. The researchers will discuss the study with undergraduate/graduate students in Kinesiology, University Sports clubs, and will also recruit using word of mouth and social media (i.e. facebook). Information provided to potential subjects will include: basic criteria for inclusion in the study, a brief description of the study demands, and benefits of participation (see Attachment A). Subjects will be selected using the inclusion criteria below, based on their responses to questionnaires (Attachments B, E). Note that female subjects will not be included. We have a track record of incorporating females when feasible. However, it is well known that estrogen levels, and therefore menstrual phase, impact muscle recovery, the chief variable in this study. So, the cost of additional analyses and design requirements of this particular project prevent us from including females.

Inclusion Criteria

To be eligible for study participation, each subject must meet the following criteria:

- Age and Sex: Males: 18-45 years
- Subjects must not have performed resistance exercise (RE) or been a part of a resistance training program within the last 3 months.
- NSAID use: Subjects will not have consumed NSAID's within the previous 7 days.
- Subjects are willing and able to give written informed consent, and to understand, participate and comply with the study requirements
- Health: Characterized as "low risk" for exercise complications using criteria from the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription (9th Ed., ACSM, 2014). Low risk is characterized by the ACSM as individuals "who are asymptomatic and register no more than one risk factor threshold" from the list below:
 - Age: Males > 45 yrs
 - Family History: Myocardial infarction, coronary revascularization, or sudden death before 55 years of age in father or other male first-degree

relative, or before 65 years of age in mother or other female first-degree relative.

- Cigarette Smoking: Current cigarette smoker or those who quit within the previous 6 months.
- Hypertension: Systolic blood pressure ≥ 140 mmHg or diastolic ≥ 90 mmHg, confirmed by measurements on at least two separate occasions, or on antihypertensive medication.
- Dyslipidemia: Low-density lipoprotein (LDL) cholesterol > 130 mg/dl or high-density lipoprotein (HDL) < 40 mg/dl, or on lipid-lowering medication. If total serum cholesterol is all that is available use > 200 mg/dl rather than LDL ≥ 130 mg/dl.
- Impaired fasting glucose: Fasting glucose ≥ 100 mg/dl confirmed by measurements on at least two separate occasions
- Obesity: Body mass index > 30 kg/m²
- Sedentary lifestyle: Persons not participating in a regular exercise program or not meeting the minimal physical activity recommendations from the U.S. Surgeon General's Report
- High-serum HDL Cholesterol: This is considered a "negative risk factor", so 1 of the above risk factors can be subtracted if HDL > 60 mg/dl

As recommended by the ACSM, we will use the AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire to identify the above criteria above (ACSM, 2014, p. 25; see Attachment B).

Study Design

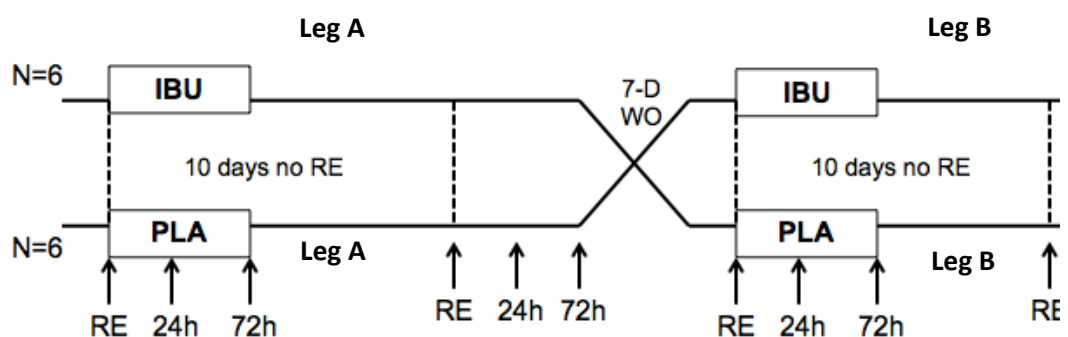
General Description

The intent of this design is to determine whether short-term Ibuprofen use during a critical window of recovery (72 hours) influences longer-term prophylactic adaptations. The proposed project will implement a randomized, double blind, placebo-controlled, crossover design whereby subjects will perform unilateral leg-extension resistance exercise. For each subject, one leg will be assigned to Placebo whereas the contralateral leg will be assigned Ibuprofen (treatments will be administered for 3 days following the initial session of unaccustomed exercise). For example, approximately 7 days following preliminary testing, 6 subjects will perform one-legged resistance exercise and receive Placebo capsules whereas 6 subjects will perform one-legged resistance exercise and receive Ibuprofen (Leg A – Exercise Session 1). The same leg will undergo identical exercise 10 days later, without treatment (Leg A – Exercise Session 2). Recovery will be assessed following each session. 10 days following the Leg A – Exercise Session 2, the opposite leg will perform one-legged resistance exercise and receive the opposite treatment (Leg B – Session 1). Leg B will then perform identical exercise 10 days later,

again without treatment (Leg B – Exercise Session 2). A schematic of the general study design is displayed in Figure 1.

Subjects will receive the treatment in intermittent doses for 72 hours with treatments commencing on the morning of the first resistance exercise trial. The same leg will be tested again 10 days later allowing for full muscle recovery, for blood markers to return to baseline, and for Ibuprofen to ‘wash-out’ so that it does not affect muscle recovery following the second session of knee extensions. This will allow us to profile the prophylactic effect without the confounding acute impact of Ibuprofen.

Figure 1: Study Design



RE= resistance exercise; Recovery will be assessed at 24 and 72 hrs following each RE.

The study will consist of a total of sixteen (16) laboratory visits totaling approximately 11 hours of commitment (in addition to completing dietary records). Specifically, subjects will report to the laboratory for one pre-testing trial, three familiarization trials, four resistance exercise trials, and eight follow-up trials. Detailed information for each of these trials is provided below:

Pre-testing 1 (60 min):

- Informed Consent – Before testing is initiated, subjects will be given consent forms to read and sign that provide a comprehensive description of the study, the risks and benefits associated with the study, and the ways in which confidentiality will be maintained (see Informed Consent).

- Body Mass and Height - Subjects will have their body weight measured to the nearest 0.2 kg, and height measured to the nearest 0.5 cm.
- VO_{2max} - During this assessment, subjects will perform a graded exercise test to determine their maximal oxygen uptake (VO_{2max}). Subjects will ride a cycle ergometer at a self-selected workload estimated as “a comfortable, but not easy pace for a 1-hour ride”. Workload will be increased by 25 W every 2 minutes until subjects voluntarily request to stop due to fatigue or are unable to continue at a cadence >50 rpm. Oxygen uptake will be assessed at each stage during this test. VO_{2max} will be assessed directly from data obtained during the test and used as a descriptive characteristic.
- Skeletal Muscle Function Familiarization – Peak isokinetic concentric muscle force and muscle fatigability will be assessed following the VO_{2max} . This test will be conducted using a Biodex muscle function device (Biodex Medical Systems Inc., Shirley NY). Muscle function will be assessed by having subjects push as hard as possible against a shin pad that is connected to an electronic device that controls speed of movement through the leg-extension. 10 continuous leg-extension repetitions will be performed. Note: Only 10 repetitions will be performed to minimize muscle adaptation to practice. Actual testing will include 30 repetitions. The purpose of this session is to familiarize the subjects with the equipment and procedures to minimize learning and improve reliability during the experimental trials.

Familiarization Trials (n =3; 15 minutes each):

- Skeletal Muscle Function - Peak isokinetic concentric muscle force and fatigability will be assessed following a standardized treadmill warm-up (3.5 mph; 5 min). See above for details. When combined with the initial skeletal muscle function familiarization test described above, each leg will receive two familiarization trials.

Resistance Exercise Trials (n=4; 60 minutes each):

- Muscle Soreness - Soreness ratings will be obtained using a 100mm visual analog scale, with 0 indicating no muscle soreness and 100 indicating impaired movement due to muscle soreness. Subjects will also be asked to complete the scale immediately following ascending, and descending a flight of stairs at normal walking speed.
- Blood Sample – Following 5 min of seated rest, fasting venous blood samples will be obtained from an antecubital vein. Approximately 10 ml of whole blood will be obtained at each blood draw, and centrifuged at 7000 rpm to remove the serum portion of the blood. Serum will be stored in a -80°C freezer for later analysis.

- Skeletal Muscle Function - Peak muscle force and fatigability will be assessed following a standardized treadmill warm-up (3.5 mph; 5 min). See above for details.
- Resistance Exercise (RE) – The RE will consist of 100 unilateral knee extensions performed at a fast speed (180 degrees per second) and 100 unilateral knee extensions performed at a slow speed (30 degrees per second). The knee extensions will be performed in sets of 10 (10 repetitions performed within each set) with a 60 second rest in between sets. Subjects will be split into two groups and randomly selected to perform the resistance exercise first with either their dominant or non-dominant leg. The RE protocol is in line with previous literature (1,2,6).

Follow-Up Visits (n=8; 30 min each):

- Muscle Soreness - Soreness ratings will be obtained using a 100mm visual analog scale, with 0 indicating no muscle soreness and 100 indicating impaired movement due to muscle soreness.
- Blood Sample – Fasting venous blood samples will be obtained from an antecubital vein.
- Skeletal Muscle Function - Peak muscle force and fatigability will be assessed following a standardized treadmill warm-up (3.5 mph; 5 min). See above for details.

Study Treatments

As outlined above, for each subject, one leg will be assigned to Placebo whereas the opposite leg will be assigned to Ibuprofen. Subjects will ingest their respective treatment capsules with water 3 x daily for 3 d, starting ~45 min prior to the initial RE test (upon arrival in the laboratory), and in subsequent 6 hour time increments for the next 72 hours.

The subjects will send a photo or text to the investigators immediately upon consumption of each capsule taken outside of the laboratory as evidence of consumption.

- Placebo: Maltodextrin-filled capsules (carbohydrate)
- Ibuprofen: The maximum daily over the counter dosage of Ibuprofen (2400mg/day) concealed in capsules matching placebo

Dietary and Exercise Controls

Subjects will be asked to keep a dietary log (see Attachment C) beginning 24 hours prior to the first RE session and for the next three days following the trial (4 days total). Upon

returning in 72 hours for a follow-up visit, subjects will submit their initial diet records and be provided with a copy to use as a template to replicate their diet for the same four days surrounding each of the next 3 RE sessions.

Subjects will consume their final 'self-selected' meal no less than 12 hours prior to the RE sessions and follow-up visits (i.e. dinner on the evening prior to testing). Subjects will also be provided with a 500 kilocalories of Ensure Active following each RE sessions (4 feedings). Subjects will be instructed to consume the feeding 60 min of RE trial completion and then to refrain from any food or beverage intake until 4 hours following RE trial completion.

Subjects will also be instructed to abstain from alcohol and caffeine for 24 and 12 hours, respectively, prior to each RE trial and follow-up visit.

Subjects will be instructed to refrain from heavy exercise for 72 hours prior to the RE trials as well as the 72 hours following each RE trial. Subjects will record all physical activity performed throughout the duration of the study, beginning with 72 hours prior to the initial RE trial (40 d) (see Attachment D). Subjects will also be instructed to abstain from the use of any supplements to enhance recovery throughout the duration of the study.

Dependent Measurements

All dependent measures will be assessed immediately prior to each RE trial and again 24 hours and 72 hours following each RE trial.

- Skeletal Muscle Function - Peak isokinetic concentric muscle force and fatigability will be assessed with BioDex. See above for details.
- Muscle Soreness - Soreness ratings will be obtained using a 100mm visual analog scale will be obtained immediately following ascending, and descending a flight of stairs at normal walking speed.
- Biomarkers – Blood samples will be analyzed for serum biomarkers of inflammation [(Interleukin-6 (IL-6) and TNF-alpha (TNF-a)] and muscle membrane disruption [creatine kinase (CK) and Brain Derived Neurotropic Factor (BDNF)]

Risks

Blood Sampling

The risks of blood sampling using venipuncture include possible mild bruising, and the risk of transfer of blood-borne pathogens, as well as possible risks of infection or skin irritation. These risks are considered to be minimal, and all safety precautions for handling blood samples will be followed according to OSHA protocols, including: investigators

will wear latex gloves at all times during blood sampling and testing. A sharps container lined with a biohazard bag will be used for all sharp objects involved in the blood sampling; all other materials (i.e. gloves, gauze pads, etc.) used during the sampling will be put in a separate waste disposal unit lined with a biohazard bag. Both investigators who will be involved in blood draws (and handling of blood) have been trained in these phlebotomy techniques, and completed JMU blood-borne pathogen training. The total amount of blood obtained during this study is approximately 120 ml. For reference, this amount is ~ 1/3 of a can of soda, or 25% of the amount given when donating blood in a single session (approximately 1 pint, or 473 ml). This amount of blood is the minimum required to perform the assays for CK, BDNF, IL-6 and TNF- α and to assess the time-course changes in these variables as a result of the exercise sessions. Subjects will be instructed to refrain from donating blood during the study period.

Skeletal Muscle Function

The risks of BioDex muscle function testing include soreness from exertion 24-48 hours post and potential lightheadedness or loss of consciousness if correct form is not utilized. Participants will be instructed in correct form and breathing techniques prior to testing.

VO_{2max} test and Resistance Exercise Trials

According to the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription, the risk associated with heavy exercise for individuals categorized as "low risk" is very minimal, and physician supervision is not necessary. The conditions that the exercise sessions are to take place are likely safer than the typical exercise environments of the subjects. Any subjects who do not meet the ACSM criteria for "low risk" will not be allowed to participate in the study. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel. In addition, at least one of the listed investigators will be present during the exercise sessions, and both are CPR certified.

The resistance exercise trials will likely evoke a large amount of muscle soreness that may persist for up to 7 d following exercise. The amount of soreness is similar to that experienced following unaccustomed vigorous physical activity (e.g. descending on a hike, playing a game of basketball, football, ultimate Frisbee, etc.)

Ibuprofen

The use of Ibuprofen may be associated with an increased risk of gastrointestinal (GI) events such as bleeding, ulcers within the stomach, and heart attack. However, serious GI reactions and CV risks are extremely low given the 3-day dosing regimen in this study. These events are more likely to occur if you have a history of ulcers, partake in drinking

and smoking regularly, are over the age of 60, are on blood thinners or blood pressure medication. Other possible side effects include rash, flare of asthma, temporary vision changes, dizziness, abdominal pain, upset stomach, burping, bloating, cloudy urine, diarrhea, decrease in urine production, constipation, heartburn, indigestion, and shortness of breath (8). Another possible risk is drug interaction with other medications such as increased bleeding risk if taken with anticoagulants, increased serum lithium levels, increased toxicity of methotrexate, or decreased effectiveness of diuretics. The research participants will be strongly encouraged to communicate any suspected or observed side effects (whether it is listed above or not) of the treatments with one of the investigators or Dr. Diduch.

Benefits

All participants will gain valuable knowledge about their own muscular physiology and information regarding the potential influence of Ibuprofen on adaptations to unaccustomed exercise. Subjects will also receive a free VO_{2max} test (valued at ~\$150) and subjects that complete all phases of data collection will be entered into 3 random drawings for \$125.

Data Analysis

This study will utilize a counterbalanced, between-subject design to examine the effects of Ibuprofen on prophylactic adaptations to exercise, compared to placebo. Dependent measurements will be examined with (treatment x time) ANOVAs. It is hypothesized that Ibuprofen will interfere with prophylactic adaptations to exercise, thus leading to larger amounts of muscle soreness, membrane disruption, and decrements in muscle function following the second treadmill session, compared to placebo. Statistical significance will be set at $p \leq 0.05$ for these analyses.

Data Handling

Participation in this research will not be completely anonymous due to the inevitable familiarity of the research team with some of the subjects. However, all subjects will be assigned an individual identification number to ensure that the data remains confidential. All files will be coded with the identification number. Coding sheets with participants' names and corresponding identification numbers along with consent forms will be kept, indefinitely, in a locked filing cabinet by Dr. Luden separate from the data files. All hard copies of data (coded with ID number) will be stored, indefinitely, separately in locked file cabinets in the Human Performance Lab. Electronic data and files will be stored, indefinitely, on a password-protected computer, and will only contain de-identified information. Only the identification number will be entered into the computer when creating data spreadsheets and therefore subject's names will not be available to those analyzing and interpreting the data.

Reporting Procedures

The results from this study may be presented at physiological conferences and in professional journals. Participants will not be identified in reports, and data from individual subjects will not be reported in a manner that would render it possible for participants to be identified. Target audiences for these results include, but are not limited to, JMU faculty, peer review professional journals, and physiology conference participants. In addition, the final aggregate results of the study will be made available to each of the subjects upon request.

Vulnerable Population

Data will not be gathered on minors, prisoners, pregnant women, fetusus, neonates, cognitively impaired persons, or other protected or potentially vulnerable populations.

Deception

No deception will be used.

Experience of the Researchers

Nicholas D. Luden, Ph.D. is an Assistant Professor of Exercise Physiology and Interim Director of JMU's Human Performance Laboratory. He has published >15 peer-reviewed manuscripts in the field of exercise physiology. His primary research interests revolve around skeletal muscle function and how it can be optimized using training and/or nutritional strategies. He has accumulated a substantial amount of both basic and applied laboratory experience over the past decade, the majority of which has been gained while conducting research on endurance athletes.

Jessica G. Ehrbar is a first-year graduate student in Exercise Physiology and a recent graduate of our undergraduate Kinesiology program. Over the past couple years, she has gained significant research experience through volunteer and practicum hours performed in our laboratory.

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Attachment A

James Madison University Department of Kinesiology Informed Consent

Purpose

I, _____, hereby agree on _____ (date) to participate in the research project conducted by Dr. Nick Luden and Jessica Ehrbar from James Madison University titled “The Effects of Ibuprofen on Prophylactic Adaptations to Heavy Exercise”

The primary goal of this study is to examine if Ibuprofen use affects muscle adaptations to heavy resistance exercise.

Subject Responsibility

I understand that I will undergo the following testing in the study:

You will be asked to visit the Human Performance Laboratory (Godwin 2009) a total of 16 times and the James Madison University Health Center to see Dr. Diduch for a health screening. Specifically, you will be asked to report to the James Madison University Health Center to be health screened prior to participation and to receive a prescription for the Ibuprofen and placebo treatments to be filled at the James Madison University Health Center Pharmacy (at no cost). Also, you will report to the laboratory for one preliminary trial, three familiarization trials, four experimental trials, and eight follow up visits 24 and 72 hours after the experimental trials. The preliminary tests will require approximately 60 minutes, familiarization trials will require approximately 15 minutes, experimental trials will require approximately 60 minutes, and the eight follow up trials will require approximately 30 minutes, for a total time commitment of approximately 11 hours in addition to completing dietary and physical activity records. Detailed information for each of these trials is provided below:

Preliminary Testing

Before any physical evaluation is given, you will be asked to complete screening forms and an informed consent, to ensure that you meet the study criteria, that you do not have any risk factors for heavy exercise, and that you do not have any known allergies to any non-steroidal anti-inflammatory drugs. In the process of filling out these forms, you will be asked to share information regarding your general health and lifestyle with the researchers. If you meet the criteria for the study, the researchers will measure your height and weight and you will perform a cardiorespiratory fitness test. During this assessment, an exercise test will be conducted to determine your maximal oxygen uptake (VO_{2max}). To do this, you will ride a stationary cycle at an initial workload that is ‘fairly easy’. Workload will be increased by 25 watts every 2 minutes during the test. You will be encouraged to continue to cycle until you request to stop due to fatigue or are

unable to continue at a cadence >50 revolutions per minute. After the VO_{2max} test, a test measuring muscle function will be performed. This test will be conducted using a Biodex muscle function device (Biodex Medical Systems Inc., Shirley NY). Peak unilateral isokinetic force (power) and fatigability will be assessed by having you push as hard as possible against a shin pad that is connected to an electronic device that controls speed of movement through the leg-extension. You will perform a trial comprised of 10 continuous leg-extension repetitions. The purpose of this session is to familiarize you with the equipment and procedures to minimize learning and improve reliability during the experimental trials.

Familiarization Trials

You will then be asked to return several days following the initial pre-testing to perform another Biodex familiarization session. Two additional familiarization Biodex sessions will be performed by your opposite leg prior to the second phase of the study.

Experimental Trials

Following preliminary/familiarization testing, you will be asked to perform four total trials of unilateral knee extensions (two trials for each leg). The two trials performed with the same leg will be separated by 10 days and the two sets of trials will be separated by 7 days.

Resistance Exercise: The RE will consist of 100 one-legged knee extensions performed at a fast speed (180 degrees per second) and 100 one-legged knee extensions performed at a slow speed (30 degrees per second). The knee extensions will be performed in sets of 10 (10 repetitions performed within each set) with a 60 second rest in between sets.

Muscle Soreness: You will evaluate your muscle soreness using a visual scale with 0 indicating (i.e.) no muscle soreness and 100 indicating impaired movement due to muscle soreness. This will be done before each of the experimental trials.

Blood Sample: Following 5 minutes of rest, blood samples will be obtained. Approximately 10 ml of whole blood will be obtained at each blood draw. This will be performed before each RE trial.

Muscle Function Test (Biodex Isokinetic Dynamometer): Following a 5-minute treadmill warm-up (walking), you will be asked to perform 30 maximal one-legged leg extensions.

Follow-Up Visits

You will be asked to return to the lab 24 and 72 hours following each resistance exercise trial for muscle function and soreness testing, and blood sampling.

Muscle Soreness: You will evaluate your muscle soreness using a visual scale with 0 indicating (i.e.) no muscle soreness and 100 indicating impaired movement due to muscle soreness. This will be done before each of the experimental trials.

Blood Sample: Following 5 minutes of rest, blood samples will be obtained. Approximately 10 ml of whole blood will be obtained at each blood draw. This will be performed before each RE trial.

Muscle Function Test (Biodex Isokinetic Dynamometer): Following a 5-minute treadmill warm-up (walking), you will be asked to perform 30 maximal one-legged leg extensions.

Study Treatments

Approximately 45 minutes before entering the lab for the resistance exercise trial, you will be required to consume 800mg of either Ibuprofen or Placebo. Every six hours for the next 72 hours, 800mg of Ibuprofen will be ingested, for a total of 9 doses of Placebo and 9 doses of Ibuprofen over the course of the study. You will be required to send a text or picture for verification to the researcher immediately following each dose.

Dietary, Exercise, and Medication Controls

You will be asked to complete a diet record for 4 days beginning 24 hours prior to the initial treadmill trial. You will submit your initial diet records the morning of your first 72 hours follow-up trial (diet records from previous 4 days). A copy of your initial dietary records will then be provided, which you will be asked to use as a template when replicating your dietary habits during the 4 days around the second treadmill trial (24 hours prior and 72 hours after).

You will consume a final 'self-selected' meal no less than 12 hours prior to the start of RE trials and follow-up visits (i.e. dinner on the evening prior to testing). You will also be provided with a 500 kilocalories of Ensure Active following each RE sessions (4 feedings). You will be instructed to consume the feeding 60 minutes of RE trial completion and then to refrain from any food or beverage intake until 4 hours following RE trial completion..

You will also be instructed to abstain from alcohol and caffeine for 24 and 12 hours, respectively, prior to each resistance exercise (RE) trial and follow-up visit.

You will be instructed to refrain from heavy exercise for 72 hours prior to the RE trials as well as the 72 hours following each RE trial. All physical activity performed throughout the duration of the study will be recorded, beginning with 72 hours prior to the initial treadmill trial (40 days). You will also be instructed to abstain from the use of any supplements to enhance recovery throughout the duration of the study.

You will be instructed to refrain from consumption of other nonsteroidal drugs (e.g. ibuprofen, advil, motrin, aleve, naproxen) starting 4 days prior to the initial trial and for the duration of the project.

Risks:

VO_{2max} test and Resistance Exercise Trials

You are expected to be honest about disclosing all known risk factors to the researcher. According to the American College of Sports Medicine, the risks associated with maximal exercise/testing for healthy individuals are very minimal. If you do not meet the criteria for “low risk”, you will not be allowed to participate in the study. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel. In addition, each of the investigators is CPR certified.

The resistance exercise will likely evoke a large amount of muscle soreness that may persist for up to 7 days following exercise. The amount of soreness is similar to that experienced following unaccustomed vigorous physical activity (e.g. descending on a hike, playing a game of basketball, football, ultimate Frisbee, etc.) The degree of soreness may vary between individuals based upon their responses to muscle damage and recovery.

Blood Sampling

The risks of blood sampling using venipuncture include possible mild bruising, and the risk of transfer of blood-borne pathogens, as well as possible risks of infection or skin irritation. These risks are considered to be minimal, and all safety precautions for handling blood samples will be followed according to OSHA protocols, including: investigators will wear latex gloves at all times during blood sampling and testing. A sharps container lined with a biohazard bag will be used for all sharp objects involved in the blood sampling; all other materials (i.e. gloves, gauze pads, etc.) used during the sampling will be put in a separate waste disposal unit lined with a biohazard bag. All trash bags will be taken to Rockingham Memorial Hospital for disposal. Both investigators who will be involved in blood draws (and handling of blood) have been trained in these phlebotomy techniques, and completed JMU blood-borne pathogen training. The total amount of blood obtained during this study is approximately 120 ml. For reference, this amount is ~ 1/3 of a can of soda, or 25% of the amount given when donating blood in a single session (approximately 1 pint, or 473 ml). This amount of blood is the minimum required to perform the assays for CK, BDNF, IL-6 and TNF- α and to assess the time-course changes in these variables as a result of the exercise sessions. Subjects will be instructed to refrain from donating blood during the study period.

Skeletal Muscle Function

The risks of BioDex muscle function testing include soreness from exertion 24-48 hours post and potential lightheadedness or loss of consciousness if correct form is not utilized. Participants will be instructed in correct form and breathing techniques prior to testing.

Ibuprofen Use

The use of Ibuprofen may be associated with an increased risk of gastrointestinal (GI) events such as bleeding, ulcers within the stomach, and heart attack. However, serious GI reactions and CV risks are extremely low given the 3-day dosing regimen in this study. These events are more likely to occur if you have a history of ulcers, partake in drinking and smoking regularly, are over the age of 60, are on blood thinners or blood pressure medication. Other possible side effects include rash, flare of asthma, temporary vision changes, dizziness, abdominal pain, upset stomach, burping, bloating, cloudy urine, diarrhea, decrease in urine production, constipation, heartburn, indigestion, and shortness of breath (8). Another possible risk is drug interaction with other medications such as increased bleeding risk if taken with anticoagulants, increased serum lithium levels, increased toxicity of methotrexate, or decreased effectiveness of diuretics. You are strongly encouraged to communicate any suspected or observed side effects (whether it is listed above or not) of the treatments with one of the investigators or Dr. Diduch.

Benefits

You will gain valuable knowledge about your own muscular physiology and information regarding the potential influence of Ibuprofen on adaptations to unaccustomed exercise. You will also receive a free VO_{2max} test (valued at ~\$150) and will be entered into 3 random drawings for \$125, if all phases of data collection are completed.

Inquiries

You may have questions or concerns during the time of your participation in this study, or after its completion. If you have any questions about the study, contact Nicholas D. Luden, Ph.D. at ludennd@jmu.edu or by phone at 540-568-4068.

Questions about Your Rights as a Research Subject

Dr. David Cockley
Chair, Institutional Review Board
James Madison University
(540) 568-2834
cocklede@jmu.edu

Confidentiality

All data and results will be kept confidential. You will be assigned an identification code. At no time will your name be identified with your individual data. The researcher retains the right to use and publish non-identifiable data. All data will be kept secured in a locked cabinet. Final aggregate results will be made available to you upon request.

Freedom of Consent

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

Name of Subject (Printed)

Name of Researcher (Printed)

Name of Subject (Signed)

Name of Researcher (Signed)

Date

Date

Attachment B

AHA/ACSM Health/Fitness Facility Pre-participation Screening QuestionnaireAssess your health status by marking all *true* statements***History***

You have had:

- a heart attack
- heart surgery
- cardiac catheterization
- coronary angioplasty (PTCA)
- pacemaker/implantable cardiac
- defibrillator/rhythm disturbance
- heart valve disease
- heart failure
- heart transplantation
- congenital heart disease

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a **medically qualified**

Symptoms

- You experience chest discomfort with exertion
- You experience unreasonable breathlessness
- You experience dizziness, fainting, or blackouts
- You take heart medications

Other Health Issues

- You have diabetes
- You have asthma or other lung disease
- You have burning or cramping sensation in your lower legs when walking short distances
- You have musculoskeletal problems that limit your physical activity
- You have concerns about the safety of exercise
- You take prescription medication(s)

Cardiovascular risk factors

- You are a man older than 45 years
- You smoke, or quit smoking within the previous 6 mo.
- Your blood pressure is > 140/90 mmHg
- You do not know your blood pressure
- You take blood pressure medication
- Your blood cholesterol level is > 200 mg/dl
- You do not know your cholesterol level
- You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)
- You are physically inactive (i.e. you get < 30 minutes of

If you marked two or more of the statements in this section, you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a **professionally qualified exercise staff** to guide your exercise program.

_____ physical activity on at least 3 days of the week)
_____ You are > 20 pounds overweight

_____ None of the above

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.

Attachment C

24-HOUR DIET RECORD

Subject number _____

Date _____ Day of Week _____

Time	Food and/or Drink	Method of Preparation	Quantity Consumed	Brand Name

Adapted From: Lee RD, Nieman DC. *Nutritional Assessment*. 2nd ed. United States of America: Mosby; 1996.

Intensity Scale

6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

Attachment E

Subject Prescreening Information

Please Complete the Following:

Age: _____ years

Height _____ Weight _____ Blood Pressure _____

Typical Exercise Habits over the Past 3 Months:

Average number of days of exercise per week _____

Average number of hours of exercise per week _____

Type of Exercise _____

Do you have a muscle or joint injury/condition that precludes the completion of the strenuous unilateral leg extension protocol? If yes, please explain.

Are you allergic to Aspirin or any Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as Ibuprofen, Motrin, Advil, etc.,? Have you taken any within the past 7 days?

Are you currently taking any prescription, or OTC medicine? If yes, please list medications.

Name of Subject (Printed)

B. Kent Diduch, M.D. (Printed)

Name of Subject (Signed)

B. Kent Diduch, M.D. (Signed)

Date

Date

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