IMPACT OF IBUPROFEN ON THE BONE RESPONSE TO SIMULATED RESISTANCE TRAINING

A Senior Scholars Thesis

by

STUART SOLOMON

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2011

Major: Molecular and Experimental Nutrition
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Approved by:

Research Advisor: Susan Bloomfield
Director for Honors and Undergraduate Research: Sumana Datta

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ABSTRACT

Impact of Ibuprofen on the Bone Response to Simulated Resistance Training.
(April 2011)

Stuart Solomon
Department of Agriculture and Life Sciences
Texas A&M University

Research Advisor: Dr. Susan Bloomfield
Department of Health and Kinesiology

The purpose of this research project was to determine the overall effects of ibuprofen on bone formation in response to simulated resistance training in adult female rats.

Ibuprofen is a common and generally safe non-steroidal anti-inflammatory drug (NSAID) that is used to treat musculoskeletal pain and inflammation. The focus of this study was to understand how the timing of ibuprofen administration in relation to simulated resistance training (SRT) affects the bone response to the training. Young adult female Sprague Dawley rats (n=15) were acclimated to a purified rat diet for 4 weeks. The animals were split randomly into three groups consisting of placebo before and after training (n=6), ibuprofen before training/placebo after training (n=4) and placebo before training/ibuprofen after training (n=5). Each rat underwent simulated resistance training every other day, for a total of 9 exercise sessions. In vivo bone scans of the proximal and midshaft tibia were taken before and after treatment in both groups by peripheral quantitative computed tomography (pQCT). Data on bone mineral density (BMD) and total area of both the proximal tibia and midshaft tibia were acquired. Serum
deoxypyridinoline cross-links (DPD), a specific marker of bone resorption, was measured to evaluate possible resorption activity in response to training and ibuprofen administration. There was no significant difference between groups in total vBMD, cancellous vBMD, and cortical vBMD of the proximal tibia, and no significant difference between groups in total vBMD and total area of the midshaft area of the tibia. However, there was a significant average percent increase in bone density and area in both tibia regions for all groups in response to the simulated resistance training. Serum DPD levels were not significantly different across groups. These preliminary data do not reveal significant effects on bone due to ibuprofen and the timing of its administration nor any differences in resorptive activity, but do illustrate a robust bone response to SRT.
## NOMENCLATURE

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>DPD</td>
<td>Deoxypyridinoline</td>
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<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Macrophage stimulating factor</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>Prostaglandin E$_2$</td>
</tr>
<tr>
<td>pQCT</td>
<td>Peripheral quantitative computerized topography</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor activator for nuclear factor $\kappa$ B</td>
</tr>
<tr>
<td>SRT</td>
<td>Simulated resistance training</td>
</tr>
<tr>
<td>TNF-$\alpha$</td>
<td>Tumor necrosis factor</td>
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Ibuprofen is a common over the counter non-steroidal anti-inflammatory drug (NSAID) that is used by millions of people worldwide to treat musculoskeletal symptoms such as sore muscles and pain from arthritis. Ibuprofen is commonly associated with exercise, as people use the drug to attenuate the small aches and pains that are caused by vigorous physical activity. Exercise and weight bearing activity is an important factor in achieving optimal bone health, as stress on bone that occurs during weight bearing activities instigate a repairing bone response that ultimately leads to increased bone density.\(^{(1)}\)

Surprisingly, the relationship between such a commonly used drug such as ibuprofen and the effects on this bone response to exercise has not yet been studied extensively.

When an anabolic stimulus is applied to bone, such as what occurs during strenuous weight bearing exercise, an important regulatory enzyme called cyclooxygenase-2 (COX-2) is activated. COX-2 in turn synthesizes prostaglandin E\(_2\) (PGE\(_2\)) as a result of the initial stimulus. Ibuprofen works by inhibiting the action of COX-2. Past research on Ibuprofen has produced data that suggests the anabolic response to bone from exercise is reduced after a single treatment of exercise. It’s clear that PGE\(_2\) plays an important role in mechanotransduction (how bone translates a mechanical stimulation into a biochemical signal).\(^{(2,3,4)}\) Past research has attempted to identify explanations for the

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This thesis follows the style of *Journal of Bone and Mineral Research*. 
mechanism on how this occurs and how Ibuprofen might alter this process\(^5\). One problem is that past studies have focused on an acute treatment of some type of weight bearing load on the bone, while the effects due to a chronic and more physiological load treatment are still mostly unknown.\(^6\)

In addition to the acute stimulus on bone and the resulting release of PGE\(_2\), there is an additional factor (among others) in the response of bone to more chronic stressors. Inflammatory cytokines are released by bone and muscle cells in response to the cellular damage incurred by physical exercise and weight bearing activity.\(^7,8,9\) It is believed that overtime; these inflammatory cytokines can accumulate in response to chronic training, resulting in a resorptive effect on bone.\(^5,10,11\) Even though past research has exhibited an overall gain in bone when subjects perform bone stimulating exercise, there still may be a measurable amount of bone resorption occurring as well.\(^9,12\) Therefore, the anti-inflammatory power of Ibuprofen in the chronically trained model may reduce the amount of accumulated inflammatory cytokines and resulting bone resorption, maximizing positive bone gains due to exercise.\(^5\)

**Prostaglandin E\(_2\) and cyclooxygenase**

PGE\(_2\) has a dual effect on bone, having the ability to cause bone formation as well as bone resorption. PGE\(_2\) achieves this effect through hormonal regulation, which is a byproduct of a network of gap junctions and dendritic connections embedded in bone that allows osteocytes buried deep within the bone to communicate with osteoblasts.
found on the surface.\textsuperscript{(13)} Due to this physiological communication, prostaglandin can stimulate osteoblasts to synthesize new bone with decreased concentrations, while higher concentrations will stimulate osteoclasts to resorb bone. Weight bearing exercise, such as lifting weights and jumping, can stimulate the release of limited amounts of prostaglandin, which is the factor that strengthens bone after exercise. The production of PGE\textsubscript{2} is catalyzed by the enzyme cyclooxygenase (COX). COX consists of two different isoforms, COX-1 and COX-2. COX-1 is expressed constantly and acts homeostatically by maintaining COX levels in bone. The COX-2 isoform is not present in most of the tissues of the body and is the most active compound in forming PGE\textsubscript{2} in a response to exercise in bone.\textsuperscript{(9)} While levels of COX-1 remain relatively constant, and COX-1 is always produced, COX-2 is produced in response to a stimulus (such as exercise) and has varying levels as a result.\textsuperscript{(2,14)}

**Mechanical load and NSAIDs: The effect on bone**

When a bone experiences a stress from a load, a large stimulus is produced over the entire cell lining surrounding the bone, which allows for the reorganization of the actin cytoskeleton in order to stimulate the production of PGE\textsubscript{2}.\textsuperscript{(15)} Previous research has shown that bone can exhibit a significant increase of PGE\textsubscript{2} in less than five minutes, while levels are brought back down to baseline within fifteen minutes after the load in cultured cells.\textsuperscript{(6,16)} The main response of bone to a mechanical load has been thought to come from this quick, initial increase in prostaglandin levels.\textsuperscript{(6,17)} In a previous study, it has been shown that when indomethacin, a non-selective COX inhibitor with a COX-
1/COX-2 ratio of 2 (meaning it inhibits COX-1 more than COX-2), is administered 3 hrs before load there is inhibition of new bone formation; however, if the indomethacin is administered 6 hours after loading there is no inhibitory response to mechanical load.\(^6\) This suggests that the COX inhibiting nature of NSAIDs can give us clues as to how PGE\(_2\) production plays a role in bone formation. It would seem that in order to have an increase in PGE\(_2\), there must be an increase in COX-2 to produce it. However, it has been shown that mechanically induced COX-2 production is not essential for loading-induced bone formation.\(^\text{18}\) It has also been shown that fluid shear strain does not cause an increase in the production of COX-2 until 30-90 minutes after the mechanical load when delivered to cultured cells.\(^\text{16,19}\)

Current research on the effects of NSAIDs on COX-2 has shown varying results based on the time of administration. When rats were given NS-398, a specific COX-2 inhibitor, 30 minutes before a mechanical load was placed on the ulna and tibia, there was not a decrease in the bone response to the acute load on the bone. However, the anticipated reduction of bone mass occurred when the NS-398 was administered three hours before the load. Because NS-398 reaches its peak serum levels after 30 minutes, it was hypothesized that the NSAID would have the same effect whether it was given three hours or 30 minutes before the mechanical load\(^\text{16}\). A recent finding in cultured cells has shown that mechanical loading on bone initiates an intracellular secretion of prostaglandin in response to the load, as opposed to stimulating prostaglandin production. This suggests that in order for prostaglandin to be suppressed, the inhibition
of the production of prostaglandin must occur a long time before the mechanical load is performed.\(^{(18)}\) However, the initial release of PGE\(_2\) that occurs within the first fifteen minutes of a mechanical load may not be the only timeframe in which prostaglandin has an anabolic effect. When a non-specific NSAID was administered six hours post-mechanical load in conjunction with NSAID administration for eight days post-load, the anabolic effect of the acute load on the bone was returned to the state measured before the load. In a group that received the same treatment but with a placebo for the eight days post-load, the anabolic response on the bone was still measurable.\(^{(6)}\) This suggests that there is a lingering window of opportunity for prostaglandin to have an anabolic effect on bone outside of the initial release.

**Inflammatory cytokines and exercise**

Inflammatory cytokines are proteins that are released by the immune system in response to a stressor. In the case of exercise, inflammatory cytokines are released by skeletal muscle when the bone is compressed and strained during a weight bearing activity.\(^{(11,20,21)}\) Inflammatory cytokines work on bone by stimulating osteoclasts to raise their activity, inducing bone resorption.\(^{(8,18)}\) The three most common inflammatory cytokines involved with bone resorption are Interleukin-1 (IL-1), Interleukin-6 (IL-6) and tumor necrosis factor (TNF-\(\alpha\)).\(^{(18,22,23)}\) The effect of these cytokines on bone are mediated by receptor activator for nuclear factor \(\kappa\) B (RANKL) and macrophage stimulating factor (M-CSF) produced by osteoblasts for osteoclastogenesis- the synthesis of new osteoclasts. IL-1 and TNF-\(\alpha\) work together to elicit a marked increase in
RANKL levels, thus showing proinflammatory regulation of osteoclast differentiation and activity through RANKL.\(^{(5,18)}\) IL-6 appears to have no effect on RANKL regulation, but has been shown to affect bone resorption indirectly.\(^{(18)}\) IL-6 stimulates bone resorption by activating a biochemical cascade of other inflammatory and proinflammatory cytokines such as IL-1 and TNF-\(\alpha\).\(^{(21)}\)

There is a marked increase in both serum TNF-\(\alpha\) and IL-1 after exercise.\(^{(21)}\) One session of intense resistance exercise in a human study yielded serum levels of IL-6 close to seven times higher than baseline one hour after the training.\(^{(3)}\) This increase in cytokines due to exercise has been thought to be a result of lipopolysaccharide leakage from the intestines, in addition to proinflammatories leaked from the muscle during exercise and contraction.\(^{(24,25)}\) Because of the powerful effect that cytokines have on bone resorption, an individual who exercises regularly will have higher cytokine levels due to the chronic inflammation, and as a result, more bone resorption. The effect of NSAIDs on exercise-induced cytokines could measurably reduce chronic levels of IL-6 that have accumulated from exercise.\(^{(26,27,28)}\)

**NSAIDs and chronic resistance training**

The use of NSAIDs to reduce inflammatory cytokine levels in an attempt to curb chronic bone resorption has been studied recently. It was demonstrated by Kohrt et al. that a positive effect on bone could be achieved when NSAIDs were administered to chronically trained individuals after exercise.\(^{(5)}\) Past research has shown that when
NSAIDs are administered after a single acute mechanical load, the effects observed are similar to the placebo group.\textsuperscript{(5)} One proposed process of how this occurs is that the positive effect on bone from the NSAIDs is due to the drug reducing unusually high inflammatory cytokine levels and suppressing the aforementioned acute burst of exercise-induced bone resorption.\textsuperscript{(5,29)}

Although NSAIDs such as Ibuprofen have been shown to reduce bone resorption from exercise-induced inflammatory cytokines, the mechanism involved in this process may be difficult to analyze, due to the many factors involved in the cytokine production from exercise. A study involving marathon runners showed a significant increase in endotoxins in a group that was administered ibuprofen.\textsuperscript{(20,25)} One proposed reason for this delineation from previous data is that Ibuprofen reduces the glomerular filtration rate (GFR) of the kidneys, reducing their ability to clear inflammatory cytokines from the body as efficiently.\textsuperscript{(31)} Because of conflicting results and their complicated mechanism of use, the study of NSAIDs such as Ibuprofen should be done under as controlled conditions as possible.

**Conclusion**

In summary, the study of the effects of Ibuprofen on bone involves several physiological processes including local and systemic factors. In order to understand this phenomenon fully, all of these physiological factors should be taken into account. It should also be explored whether or not the timing of administration of the Ibuprofen has a noticeable
difference to the effect on bone. Finally, the effects of Ibuprofen on bone in response to chronic weight bearing exercise should be compared to that of an acute treatment of weight bearing stimulus. For individuals that put regular stress on their bones due to exercise, research on a simple yet effective way to maximize bone formation will be beneficial. If a better understanding of how ibuprofen affects bone remodeling in response to exercise can be achieved, we can potentially come closer to providing a recommendation to individuals that take NSAIDs before or after exercise on when to take them to maximize bone health.
CHAPTER II

METHODS

Animal information
Young adult Sprague-Dawley rats were used in this study as an animal model to
demonstrate the effects of ibuprofen on the bone response to simulated resistance
training (SRT). Sprague-Dawley rats have consistently demonstrated a robust bone
response to voluntary and simulated resistance exercises in our past research.\(^{4,12,32}\)
Finally, due to the multiple serum analyses that were required during this study, rats,
rather than mice, were ideal because of the larger volumes of blood that can be collected
from rats.

Upon arrival, 15 female Sprague-Dawley rats, 4.5-months old at arrival, were housed at
an animal facility on Agronomy Road, two to a cage, on a 12:12 hour light:dark cycle.
After one week of acclimation, the rats were rank-ordered by body mass and then block
assigned to one of the 3 experimental groups. This block randomization strategy helps
assure groups with roughly equal mean body weight, which is important since body
mass is a strong predictor of bone mass. One group received a placebo solution (1 ml of
vehicle [methylcellulose] without ibuprofen) before resistance exercise and after
resistance exercise, one group received ibuprofen (1 ml of vehicle [methylcellulose] with
ibuprofen) before resistance exercise and the placebo after resistance exercise, and one
group received the placebo before resistance exercise and ibuprofen after resistance
exercise. Table 1 shows the groups in the experiment. Those groups that received ibuprofen were given a 30 mg/kg dose. The literature states that a dose between 10mg/kg and 30mg/kg is safe\(^{33}\). Previous research has effectively used 30 mg/kg for fracture healing studies\(^{27,30}\) and it has been stated that the ulcerogenic dose in rats is 455 mg/kg (4 daily doses)\(^{34,35}\). The animals in this study will only be receiving a dose of 30 mg/kg 3 days per week, which is well below the 455 mg/kg ulcerogenic dose.

**Table 1. Experiment Groups**

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<th>After Ex</th>
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<td>Placebo</td>
<td>Placebo</td>
</tr>
<tr>
<td>I:P</td>
<td>Ibuprofen</td>
<td>Placebo</td>
</tr>
<tr>
<td>P:I</td>
<td>Placebo</td>
<td>Ibuprofen</td>
</tr>
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</table>

Starting at roughly 5 months of age, all rats began their simulated resistance training regimen, which was performed 3 days per week. Training continued for 5 weeks, as a robust bone response after this period of time has been previously documented.\(^{4,12}\) All animals received approximately 1 ml of vehicle (methylcellulose) with or without ibuprofen by oral gavage before and after each training session.
Number of animals

9 rats per group provides adequate statistical power (0.74 – 1.00) to detect changes of 8% in total and cortical BMD and changes of 15% in cancellous BMD at the proximal tibia, as measured by pQCT. Total, cancellous and cortical BMD determined by pQCT are the key variables we track to assess loading induced changes in bone mass. We have previously determined population variance on adult rats for these pQCT-derived measures to range from 3% for cortical BMD to 11% for cancellous BMD. This thesis provides results from only the first cohort of animals in the whole study. Therefore, the statistical power to detect group differences is much lower than desirable; hence these data must be considered preliminary in nature.

Procedures

In-vivo pQCT scan: Measures of tibial bone density and geometry were taken in-vivo using our peripheral quantitative computed tomography (pQCT) device (XCT Research M Stratec; Norland Corp., Fort Atkinson, WI). pQCT scans are an ideal way to measure bone density and geometry in animals because they can be performed in vivo before and after treatment, providing a high statistical power. Another large advantage of pQCT is its ability to differentiate between cancellous and cortical bone compartments within the same slice, as opposed to 2-D methods like dual energy x-ray absorptiometry. Animals were anesthetized with inhaled isofluorane (to effect) and scan slices were taken at both the metaphyseal (3.25 3.75, 4.25, 4.75 mm distal to a reference line set at the knee joint) and diaphyseal (50% total bone length) regions, with voxel size of 100um. Total scan
time was approximately 20 minutes from time of scout view until scanning is complete. Key outcome variables include total, trabecular, and cortical volumetric bone mineral density (vBMD); total and cortical area, total bone mineral content (BMC); and cross-sectional moment of inertia (CSMI) and section modulus, which combines CSMI and vBMD data to provide an estimate of bone strength. This study reports total, trabecular and cortical vBMD of the proximal tibia and total vBMD and total area of the midshaft tibia.

Simulated resistance training: Animals received SRT sessions every other day for 3 weeks for a total of 9 SRT sessions. After the animal was anesthetized, electrodes were placed across the sciatic nerve in order to stimulate the sciatic nerve and generate a contraction of total leg musculature. The hip and knee joints were immobilized to isolate only the movement of the talocrural (ankle) joint. The foot was attached to a servomotor with measurable torque inputs and outputs, enabling us to measure the force the foot enacted on the foot plate during isometric contraction, and the amount of force placed against the foot by the foot plate during eccentric contraction. Training intensity for both types of muscle contraction was standardized for each animal at 75% of their peak isometric contraction strength. Each SRT session consisted of 4 sets of 5 contractions for a total of 20 contractions per session. Each contraction consisted of 1 isometric contraction followed immediately by a 1 second eccentric contraction. Range of motion for the eccentric contraction occurred from -40° to 40°.
Blood Draws for interleukin-6 assays: Blood draws were taken from the saphenous vein after the 3rd training session, within the first week, and 3 sessions before the final session, within the 5th week. Animals were put administered isoflurane in order to obtain the blood samples. Approximately 250µl was obtained. Rats were anesthetized with inhaled isofluorane (to effect) and a tourniquet tied around the upper leg to apply intermediate pressure; the lower leg was shaved to aid in visualizing the saphenous vein, and venous blood collected with a 26 ga needle into a microfuge tube. Twenty-four hours after the final training session (day 21), rats were anesthetized and euthanized by decapitation; cardiac serum was collected and tibia and femurs cleaned of soft tissue, wrapped in saline-soaked gauze and frozen at -80º until further analysis.

Assay for interleukin-6: Standard ELISA procedures were planned for interleukin-6 assays utilizing kits from ImmunoDiagnostics Quantikine. Duplicates of a pooled serum sample in each separate kit were to be used in each assay in order to quantitate inter-kit CV’s, in addition to the standards for the calibration curve. All samples were to be organized so that any one rat’s samples would all be assayed with one kit.

Assay for deoxypyridinoline: Deoxypyridinoline (DPD) was chosen to evaluate bone resorption in our experimental groups because DPD is a more accurate measure of specific bone resorption when compared to alternatives such as hydroxyproline(36). Free serum DPD was measured from cardiac serum collected on day 21 by ELISA assay in order to evaluate if bone resorption was occurring as a result of SRT (Microvue DPD
assay, Quidel Corp, Mountain View, CA). The intra-assay CV was ~12% and the inter-assay CV was ~9%. Samples were run as duplicates with an incubation time of 23 hours.
CHAPTER III

RESULTS

Reflection on methods

Although the animal training and administration of ibuprofen proceeded with only a few obstacles, the IL-6 ELISA assay that was originally planned for this study presented us with challenges. We were able to generate an excellent standard curve, but the values we generated for our cardiac serum samples clustered in the bottom 10% of the standard curve. As a result, we were not confident in the data for serum IL-6. Since we have very limited volumes of serum from the pre- and post-exercise time points, we could not risk re-running this assay without more serious troubleshooting. Although we are still hopeful that the IL-6 data can be generated in the future, there simply was not enough time to address the problems caused by the assay kit before these data were to be presented at Student Research Week and included in this undergraduate thesis project. Fortunately, other aspects of the study proceeded as planned, with the pQCT scans contributing compelling data. From that point, the study of this thesis project shifted from the relationship between bone gains and serum IL-6 levels to the effects of ibuprofen on bone density and geometry as measured by pQCT and any detectable bone resorption activity that be caused by transient elevation in cytokines after exercise as measured by serum DPD levels.
**pQCT results**

Figure 1 shows the total bone mineral density (vBMD) of the proximal tibia averaged from four scans of pQCT. There was not a significant difference between ibuprofen groups, but there was a significant average increase in vBMD for all groups between pre and post exercise. Figure 2 shows the vBMD of the cancellous compartment of the proximal tibia. There was not a significant difference of gain in bone between groups, but there was a significant percent increase in vBMD for all groups from pre to post exercise. Figure 3 shows the vBMD of the cortical shell of the proximal tibia between groups. There was not a significant difference between groups for the cortical shell, but a significant increase in cortical shell vBMD occurred from pre to post exercise. Figure 4 shows the total vBMD of the midshaft of the tibia. Although the three groups were not significantly different from one another, there was a significant change for all of the groups between pre and post exercise. Figure 5 shows the total area (in mm$^2$) of the midshaft of the tibia. There was not a significant difference between ibuprofen groups in tibia midshaft area, but a significant percent increase occurred from pre to post exercise.

**Deoxypydinoline results**

There were no significant differences in the serum levels of DPD between all groups, as measured on day 21 of the experiment (post exercise). Table 2 shows average DPD values of the three experimental groups.
FIG. 1. Total vBMD of the proximal tibia between ibuprofen groups. Although there was no significant difference between groups, there was a significant percentage gain in total vBMD for all of the groups between pre and post exercise.

FIG. 2. Cancellous vBMD of the proximal tibia between ibuprofen groups. Although there was no significant difference between groups, there was a significant percentage gain in cancellous vBMD for all of the groups between pre and post exercise.
FIG. 3. Cortical vBMD of the proximal tibia between ibuprofen groups. There was not a significant difference between the three groups, but a significant gain in cortical vBMD was seen from pre to post exercise.

FIG. 4. Total vBMD of the midshaft tibia between ibuprofen groups. Although there was no significant difference between groups, there was a significant percentage gain in midshaft vBMD for all of the groups between pre and post exercise.
FIG. 5. Total area of the midshaft tibia between ibuprofen groups. Although there was no significant
difference in gain in area of bone between the groups, there was a significant increase in area for all of the
groups from pre to post exercise.

Table 2. Deoxypyridinoline Levels

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<tbody>
<tr>
<td>Deoxypyridinoline (pmol/ml)</td>
<td>5.38 ± 1.78</td>
<td>5.92 ± 2.21</td>
<td>5.12 ± 1.38</td>
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Data shown as mean ± SD. No significant differences were found among the 3 group means, by
one-way ANOVA.
CHAPTER IV
DISCUSSION AND CONCLUSIONS

Discussion

This study was the first to evaluate a chronic controlled resistance training model with ibuprofen in animals. Past research has established that simulated resistance training is an effective treatment in producing a measurable change in bone density and geometry, and these results further support these previous findings\(^{12}\). PGE\(_2\) has been identified as a major contributor to bone growth in response to training\(^{2,4}\). Based on the findings from previous studies on how NSAIDs interfere with the release of PGE\(_2\) and its resultant effects on bone, we hypothesized that a more chronic training model would reveal more definitive information on how this process occurs\(^{6,16,18}\). After the 21 days of training, there was a significant gain in bone density and favorable changes in cross-section geometry for all rats in all groups. This is important, as SRT has been established as a more physiological loading model in animals. By mimicking the stresses on bone that might be seen in the real world, while having the ability to quantitatively measure the amount of torque generated by the muscles acting on the bone, we can have a more controlled bone forming treatment in addition to increasing the credibility of our findings due to the physiological relevance of the treatment.

Even though there was a marked increase in bone from pre to post training in the animals, the effects of ibuprofen that have been demonstrated in past research were not
observed in this study. Chow and Chambers revealed how a non-selective
cyclooxygenase inhibitor reduced the gains in bone provided by an acute bout of
mechanical loading\(^6\). Other studies expanded on this finding, demonstrating how COX-
2 is especially important in growth of lamellar bone by using NS-398, a selective COX-2
inhibitor\(^{16,37}\). Although we expected to observe similar results due to the mostly COX-2
inhibiting nature of ibuprofen, we could not reproduce previous data. One possible
reason for this is that the simulated resistance training was too strong of a mechanical
load, causing an overwhelming bone response that would mask any more subtle
differences that would be caused by ibuprofen. We chose 75% of the isometric
maximum contraction based on our previous procedures and a small pilot study that
preceded this experiment, but it still may have been too intense\(^{12}\). In addition, our
previous studies used an eccentric contraction ranging from -20° to 20° range of motion,
where we used -40° to 40° range of motion, resulting in higher eccentric torques, and
therefore a more robust stimulus delivered to the bone. All of these factors contributed to
an almost extreme gain in bone density and geometry, which may have masked effects
of ibuprofen that were occurring.

Another possible reason we did not observe any effects of ibuprofen in this study was
the small sample size. Due to time constraints, only the first cohort of the experimental
animals was included in this report. The second cohort, which will double the total
number of animals in the study, was completed 1 week before the submission of this
thesis. Due to the low numbers of animals, there may not have been enough statistical
power to see a significant difference in bone gains between ibuprofen groups. Finally, there was a small amount of error in administering the ibuprofen in the anesthetized animal in the first week of the study. Some of the animals may have aspirated a small amount of liquid ibuprofen during oral administration. The ibuprofen was administered by means of gavage in order to deliver the ibuprofen directly to the stomach and control time of delivery. This method was chosen to best mimic the human response to NSAIDs taken orally. About 10 days into training, these procedural errors were corrected by holding the animal in an upright position in order to prevent regurgitation of the ibuprofen resulting in inhalation. However, presumably due to first days of training and possible ibuprofen aspiration, the animals that received ibuprofen after training had a lower body weight profile than the other groups. These differences were not statistically significant, but lower body weights may have reduced potential gains in bone for the ibuprofen after exercise group. We expected this group to have a higher gain in bone when compared to the other groups.

The analysis of serum DPD levels did not produce a significantly different result, and levels of DPD in our animals were in the acceptable range for young adult female rats\(^{36}\). This result suggests that SRT with or without ibuprofen administration does not have a measurable effect on bone resorption. It is possible that because cardiac serum was used, the exercising of one leg was not enough to see a significant change in systemic levels of DPD. Perhaps a more interesting result would have been observed if serum from the local site (like the saphenous vein) was used to analyze DPD levels.
Another possible explanation for the lack of differing DPD levels is that the simulated resistance training had such a bone stimulating effect that it masked any possible detriment on the bone that would be caused by accumulating levels of IL-6 and other inflammatory cytokines. It will be interesting in the future to compare cytokine levels with the level of bone resorption markers to see if there is a relationship between the effects of simulated resistance training and accumulation of inflammatory cytokines.

**Conclusion**

Simulated resistance training in a full weight bearing rat model produces a highly potent gain in bone with a more physiological form of mechanical loading than those used in previous models. These preliminary data did not reveal significant effects on bone due to ibuprofen and the timing of its administration with several possible factors being the cause of these unanticipated results. Given the compelling previous data in the published literature, it will be important to continue to pursue interactive effects of NSAIDs and exercise on bone outcomes.
REFERENCES


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