Effects of blood vessel permeability on mechanical properties of bone under micro-gravity conditions

Robert L Drollinger

University of Nevada, Las Vegas

Follow this and additional works at: https://digitalscholarship.unlv.edu/rtds

Repository Citation
https://digitalscholarship.unlv.edu/rtds/1588

This Thesis is brought to you for free and open access by Digital Scholarship@UNLV. It has been accepted for inclusion in UNLV Retrospective Theses & Dissertations by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.
EFFECTS OF BLOOD VESSEL PERMEABILITY ON MECHANICAL PROPERTIES OF BONE UNDER MICRO-GRAVITY CONDITIONS

by

Robert L. Drollinger

Bachelor of Science in Mechanical Engineering
Minor in Biology
University of Nevada at Las Vegas
2000

A thesis submitted in partial fulfillment of the requirements for the

Master of Science Degree in Biomedical Engineering
Department of Mechanical Engineering
Howard R. Hughes College of Engineering

Graduate College
University of Nevada at Las Vegas
December 2003
Thesis Approval
The Graduate College
University of Nevada, Las Vegas

November 12, 2003

The Thesis prepared by

Robert L. Drollinger

Entitled

Effects of Blood Vessel Permeability on Mechanical Properties of Bone

Under Micro-Gravity Conditions

is approved in partial fulfillment of the requirements for the degree of

Masters of Science in Biomedical Engineering

Examination Committee Chair

Dean of the Graduate College
ABSTRACT

Effects of Blood Vessel Permeability on Mechanical Properties of Bone Under Micro Gravity Conditions

by

Robert L. Drollinger

Dr. Bingmei Fu, Examination Committee Chair
Assistant Professor of Mechanical Engineering
University of Nevada at Las Vegas

Loss of bone mass is one important physiological change observed in astronauts after medium and long-term exposure to microgravity conditions. The objective of the current study is to test the hypothesis that loss of bone mass under microgravity can be inhibited if the same micro-force distribution under earth gravity (body weight) is achieved by increasing the blood vessel permeability to increase the transcortical interstitial fluid flow. A method of exposure to bio-frequency spectrum (BFS) light (infrared to micrometer wavelength) was used to increase the blood vessel permeability. We tested the hypothesis by examining the bone mechanical properties (Young’s modulus) of rat femur and humerus for four groups of animals: i) control, ii) exposure to a bio-frequency spectrum (BFS) light, iii) tail-suspension (simulating microgravity condition), and iv) tail-suspension and exposure to a bio-frequency spectrum (BFS) light. Twenty-eight adult rats of ~ 250 g were used and kept for 50 days before sacrificing. The average Young’s moduli for each group of rat femurs are i) 1.78 GPa, ii) 2.04 GPa, iii) 2.03 GPa,
and iv) 2.04 GPa; for each group of rat humeri are i) 3.83 GPa, ii) 4.14 GPa, iii) 3.78 GPa, and iv) 3.80 GPa. These results indicate that exposure to BFS light only induces a slight increase in bone strength (Young’s modulus) for both control and tail-suspension after 50 days. To confirm our hypothesis we need to do longer-term experiments or use other methods for observing the micro structural changes in bone.
# TABLE OF CONTENTS

ABSTRACT ............................................................................................................................. iii
LIST OF FIGURES ................................................................................................................ vii
LIST OF ILLUSTRATIONS ................................................................................................ viii
LIST OF GRAPHS ................................................................................................................ ix
CHAPTER 1 INTRODUCTION ............................................................................................. 1
  1.1 Background and Significance .................................................................................... 1
    1.1.1 Bone Loss Phenomena ....................................................................................... 1
    1.1.2 Anatomy of Bone .............................................................................................. 3
      1.1.2 a) Use of the Cable Model ............................................................................ 4
      1.1.2 b) Gap Junction Model ................................................................................. 6
      1.1.2 c) Fiber-Matrix Model .................................................................................. 7
    1.1.3 Previous Methods for Preventing Bone Loss or Weakening ............................ 10
    1.1.4 New Theory for Bone Regeneration ................................................................ 12
    1.1.5 Specific Aims and Methods ............................................................................. 13
  1.2 Objectives of This Study ......................................................................................... 16
    1.2.1 Hypothesis ....................................................................................................... 16
    1.2.2 Objective of This Study .................................................................................. 16
  1.3 Definition of Terms .............................................................................................. 16

CHAPTER 2 EXPERIMENTAL DESIGN AND METHODS .................................................. 18
  2.1 General Description of Experimental Design ......................................................... 18
    2.1.1 Description of Animals Being Tested ............................................................... 19
    2.1.2 Bone Extraction ............................................................................................. 19
      2.1.2 a) Rat Preparation ....................................................................................... 19
      2.1.2 b) Femur Extraction: ................................................................................... 19
      2.1.2 c) Humeri Extraction .................................................................................. 21
    2.1.3 Bone Preservation .......................................................................................... 23
  2.2 Experiment Set-up .................................................................................................. 23
    2.2.1 Component Construction ................................................................................ 23
    2.2.2 Taping the Tail ............................................................................................... 28
    2.2.3 Biofrequency Spectrum Lamp ......................................................................... 32
    2.2.4 Tail Suspension Technique ............................................................................. 34
    2.2.5 Test Source Machine for “E” (Young’s Modulus) Test ................................... 36
      2.2.4 a) Test Resources Q100 Testing Machine .................................................... 38
  2.3 Young’s Modulus Measurement ............................................................................. 39
LIST OF FIGURES

Figure 1  Molecular Response of Bone to Unloading ....................................................... 2
Figure 2  Compact and Cancellous Bone ................................................................. 3
Figure 3  Trabecular Bone ...................................................................................... 4
Figure 4  Gap Junction Model ................................................................................. 6
Figure 5  Simplified Model of Lacunar-Canalicual Pores .................................. 8
Figure 7  The Cortical Bone .................................................................................. 13
Figure 8  Enlarged View of Lacunar-Canalicual Porosities .................................. 14
Figure 9  Cell Membrane Deformation Caused by Increased Drag .................. 15
Figure 10 Musculature of the Hind Limb of a Rat .............................................. 20
Figure 13 Lateral and Medial Views of the Forelimb of a Rat ............................... 22
Figure 14 Bending Blocks for Suspension Brackets ........................................... 24
Figure 15 Suspension Brackets with Axle and Wheels Attached ...................... 24
Figure 16 Lathing the Pulleys and Wheels from Stock ........................................ 25
Figure 17 Tail Suspension Clip with Pulley on Axle ............................................. 26
Figure 18 Complete Suspension Cage Mounted on Standard Rat Container .......... 27
Figure 19 Tail Taping Detail ................................................................................ 28
Figure 20 Design Parameters for Tail Suspension .............................................. 34
Figure 21 Force Application Over a 3 Point Bending Device ................................... 39
Figure 22 Radii Averaging .................................................................................... 41
LIST OF ILLUSTRATIONS

Illustration 1  View of Rat Suspended from Rear ............................................................... 29
Illustration 3  Cage storage of Control and BFS Groups ................................................... 31
Illustration 4  Rats being exposed to BFS lamp................................................................. 32
Illustration 5  View of Suspended Rat from the Side ......................................................... 35
Illustration 6  View of suspension device from Angle ...................................................... 36
Illustration 7  Q100 with humerus in compression for 3 Point Bending ......................... 37
Illustration 8  Close up view of bone in 3 Point Bending ................................................ 38
Illustration 9  Tethered rat trying to escape ................................................................. 51
Illustration 10 Necrotic tail. ............................................................................................... 52
Illustration 11 This illustrates the use of a rubber matting............................................... 53
LIST OF GRAPHS

Graph 1  BFS Frequency Band of Operation (Chen et al, 1995) ........................................ 33
Graph 2  Load vs. Displacement for a Femur .................................................................. 42
Graph 3  Linear Region of Load vs Displacement for Humerus (k constant = 222.8)  43
Graph 4  Young’s Modulus of Femurs ......................................................................... 43
Graph 5  Load vs. Displacement for Humerus ................................................................. 45
Graph 6  Linear Region of Load vs Displacement for Humerus ( k constant =125.4)  46
Graph 7  Young’s Modulus for Humeri ........................................................................... 46
Graph 8  Averaged Load vs. Displacement for 4 Groups ................................................. 49
CHAPTER 1

INTRODUCTION

1.1 Background and Significance

1.1.1 Bone Loss Phenomena

For many years scientists have researched the problem with bone loss due to microgravity, osteoporosis and prolonged bed rest. The problem with each of these is the unloading of bones that are normally meant for weight bearing. (1-3,5-14,16-25) These bones that suffer the greatest losses are those that normally support the body such as those of the legs, pelvic region and spine.

Under micro-gravity or zero gravity conditions there is an induction of bone loss of both trabecular and cortical envelopes. The bone loss is usually found in the weight bearing bones of the back, pelvic area and legs. The osteoclasts, which are responsible for the breakdown of bone tissue, continue to perform normally or at a faster rate in a resorption mode. The problem is found with the osteoblasts, whose job is to regenerate the bone tissue, having a decreased rate in bone formation. (2,3,8,13,14,16,24) The overall effect is the decreased density, mechanical properties and volume of the weight bearing bones. The result is the increased risk of fractures and the reduction of the bones' ability to produce blood products. (18,20,24) The same problem is prevalent in osteoporosis and osteoarthritis patients where the remodeling of the bone does not match that of the breakdown of the bone by the osteoclasts. The demineralization of the bone is
a result of the mechanical unloading of the skeletal structure during space flight and prolonged sitting or bed-rest as in elderly or lethargic people. In Skylab, (Kumei et al, 1996) astronauts had an estimated average loss of 140 mg per day in calcium during various length of space flights. Urinary levels taken from the astronauts showed elevated levels in calcium, hydroxproline and phosphorus. With the increased levels in these minerals in the urine, there is also an increased risk of stone formation within the kidneys. (Whitson et al, 1993)

![Diagram of bone response to growth hormone](image)

**Figure 1** Molecular Response of Bone to Growth Hormone During Skeletal Unloading (Heer et al, 1999)

With gravity as an agent in inducing stress, it has been found that bone formation is directly related to this stress. (6,23) In a study by Selye (1946), stress induces the adrenal secretion of cortisol as an essential component of bone remodeling. Glucocorticoid levels are increased which cause the osteoblast differentiation to increase the enhancement of
osteogenesis. In vitro introduction of the glucocorticoids inhibit osteoblast recruitment. Therefore it is important to note that by increasing stress across the bone cells the glucocorticoid levels can naturally increase and thus stimulate osteoblast recruitment. The increased recruitment of osteoblasts lead to bone formation through osteogenesis.

This thesis focuses on the development and execution of an experiment designed to address mainly the problem associated with mechanical property changes as a part of micro-gravity exposure. This experiment facilitates the use of a bio-frequency spectrum lamp or BFS lamp as a means to attempt to increase microvessel permeability to increase the shear stress to eventually strengthen the mechanical properties of the bones.

1.1.2 Anatomy of Bone Structure and Possible Mechanisms for Bone Cell Regeneration

(Zhang et al, 1997) The structure of the bone being studied can be shown in the following figures, Fig 2 and Fig 3. The approximate size a single substructure of the bone called the osteon is 200 µm.

Figure 2  Compact and Cancellous Bone
(http://training.seer.cancer.gov/module_anatomy/images/illu_compact_spongy_bone.jpg)
Taking the above figure and magnifying it to an even smaller scale one can look more directly at the lacunar and canalicular cannals within the osteon as depicted in the following figure. The size of the canalicular processes are roughly 2 μm.

Figure 3 Trabecular Bone (Zhang et al, Annuls of Biomedical Engineering 1997)

1.1.2 a) Use of the Cable Model

Zhang and coworkers used a combination of various previous researchers’ methods of the cable theory to show that SGP is related to the intracellular current through the osteocytic process by harmonic mechanical loading. (23) Their method included the capacitance of the membrane of the osteocytic processes, leakage of current through the osteoblasts and negation the atomic structure of the cellular network. The structures are depicted in the previous figures.
The use of a cable model (Zhang et al, 1997) estimates the special distribution of the intracellular electric potential and current using the alteration of the frequency of loading and the conductance of gap between the osteon to the cement line of the lumen. Live bone is more responsive to mechanical loading in the frequency range of 15-30 Hz.

Osteocytes are the bone cells encased in the bone matrix and have a length of approximately 35 μm from one gap junction to another and a diameter of approximately 0.15 μm. By varying the permeability of the gap junctions the electrical conductance of the gap correlates with that variance. The conductance of that gap seems to be a product of the number of: channels, the conductance of a single channel, and the fraction of time that it is open. When the bone is mechanically loaded it initiates a small intracellular electrical signal via the piezoelectric effect. The strain generated streaming potential (SGP) is found at the lacunar-canalicular porosity and a fiber filled annulus with an inner boundary nearly ½ of the canalicular radius. These gap junctions respond to external forces such as mechanical loading or internal signals like the transjunctional potential drop. Biddle et al 1997 believed that three mechanisms are responsible for bone formation under mechanical loading: Streaming potentials, mechanical strain, and fluid shear stress. A fourth although mentioned, piezoelectric effect, did not exhibit a high enough potential to be considered was not discussed in this article. Streaming potentials occur when the bone is bent or flexed causing movement of ionic milieu flowing over the cells. The displacement of the charge develops a local electric field that predisposes cell metabolism. Mechanical strain activates a stretching of the ion channels and other membrane associated proteins. Fluid shear stress is likely to combine the first two mechanisms and will be studied in this project.
1.1.2 b) Gap Junction Model

The interstitial fluid flow through the lacunar-canalicular gaps helps to create the cell membranes' deformations. The figure on the following page shows the theoretical gap junctions in the cell processes. These gaps are where a potential drop occurs and stimulation of the osteocytes begins.

![Gap Junction Model](image)

**Figure 4** Gap Junction Model (Zhang et al, 1997)

The pore fluid pressure and the SGP exhibit a strong dependence on frequency that is related to the length of the structure being drained and its diffusivity. By calculating the value of the ratio between the gap junction and the resistance of the cell process with length one can predict how many gap junctions are open. (23)

The behavior of the pore fluid pressure within the bone under external stresses is controlled only by the diffusivity coefficient of the bone. This coefficient relies on the permeability of the fiber matrix surrounding the osteocytic process, the viscosity of the
intercellular fluid, and the solid to fluid interaction within the lacunar-canalicular porosity. For the lacunar-canalicular system a value for \( c \) is predicted at \( 3.76 \times 10^{-2} \) m\(^3\)/sec (Zhang et al, 1997). Time is a factor when calculating the potential across the gap junction. The junctions closest to the osteoblasts are the primary regulators of the potential. This potential could be the signal for osteoblasts to start remodeling and is controlled by mechanical loading and the hydrostatic fluid forces within the bone porosities. (3,15)

1.1.2 c.) Fiber-Matrix Model

The fiber-matrix model, a theoretical model of the fluid flow and the strain generated potential in the lacunar-canalicular porosity of an osteon that is loaded perpendicularly or parallel to the osteonal axis. The model can be used to describe the fluid shear stress acting upon the cell membrane of the osteocytic process under mechanical loads. This research addressed four different sections through mathematical formulation. These four sections include: the flow in the canaliculus, determining pore pressure in the osteon, electro kinetic theory for determining the SGP and the calculation of fluid shear stress in the osteocytic process, Zeng et al (1994).

For flow in the canaliculi there are three different levels of permeability: open spacing between GAG (glycosaminoglycans) fibers, thickness of the annulus, and the largest is the permeability constant that appears to govern pore pressure.

The shear stress of the osteocytic process during a loading cycle results in a maximum value. This shear stress is a function of the fiber matrix parameters and the geometry of the canaliculus. The maximum value of the shear stress occurs where the pressure or potential gradient is at their maximum. In this case it would be at the luminal surface of
the osteon. The most likely range of the approximated shear stress value of 16-25 dynes/cm².

An attempt was made to connect the concept that mechanical loading affects interstitial fluid flow thus effecting the blood flow within the bone. By using a simplified bone model:

![Simplified Model of Lacunar-Canalicular pores](image)

**Figure 5** Simplified Model of Lacunar-Canalicular pores (Fritton et al, 2001)

By using the permeability of the lacunar-canaliculare pores at $1.5 \times 10^{14}$ mm² and that of vascular pores on a whole bone level at $5 \times 10^8$ mm², the results showed that when mechanical loads cycled at 1 Hz and 1000 microstrain, the velocity of fluid into the marrow cavity ranged from ± 0.125 microns/sec. In this study it is not clear whether the mechanical loading does or does not have a significant effect on the blood flow in the bone vessels. Although it does not show a connection between interstitial fluid flow and blood flow this study shows the importance of osteonal and bone geometry analysis as circular for studying permeability of lacunar-canaliculare pores (Fritton et al, 2001).
Using a model to simulate the excitation of osteocytes by mechanical loading, shear stresses of fluid in bone was induced in an experiment by a group of researchers in the Graduate School of the City University and School of Engineering of the City College in New York. This study showed that the osteocytes may not be overly responsive to large changes in fluid strain within the canaliculi but are responsive to small changes. The small changes used in the model corresponded with the measured changes within the fiber spacing of the canicular pore spaces that are filled with glycosaminoglycans. This suggests that high-frequency and low amplitude postural strains could be responsible for maintaining or increasing bone mass in a micro gravity or zero gravity environment. (Weinbaum et al, 1994) During rigorous exercise, a strain on the surface of the substrate remains below 0.2 %. The hypothesis tested showed that bone cell formation was directly related to the flow induced by loading of the bone through the lacunar-canicular regimes. However, it is very difficult to obtain a quantitative analysis of this phenomenon by predicting the shear stress applied to bone cells in vivo in the range from 0.8-3 N/m². Different studies have shown, however that the shear stress has had an effect on bone formation (3), and was administered at levels far exceeding those encountered by routine physical activities. You, J et al 2000 showed that a silicone membrane and a computer controlled ZETA 6104 achieved motor-driven micrometer substrate deformation. This provided the means by which the substrate could be deformed to as little as 1 μm for the proper amount of dynamic strain. This strain was verified using optical tracking markers. In this particular apparatus a 0.0001 N/m² average shear wall stress occurs at 1% strain. Using an ultrasonic flow meter the quantification of fluid flow could then be evaluated. This study determined the fluid flow
through the lacunar-canalicular regimes with a given deformation but not the forces experienced in living bone tissue under strain.

With gravity as an agent in inducing stress it has been found that bone formation is directly related to this stress. In a study by H. Selye in 1946 it was found that stress induces the adrenal secretion of cortisol as an essential component of bone remodeling. Glucocorticoid levels are increased thus increasing the osteoblast differentiation enhancing osteogenesis. In vitro introduction of the glucocorticoids inhibit osteoblast recruitment, thus it is important to note that by increasing stress across the bone cells the glucocorticoid levels can naturally increase thus stimulate osteoblast recruitment and bone formation through osteogenesis.

1.1.3 Previous Methods for Preventing Bone Loss or Weakening

During space flight, the exercise program that is currently used prevents loss of muscle mass but does nothing for the loss of bone mass. One study tested the hypothesis that bone mass can be maintained if a certain mechanical impulse is applied for short periods of time during space flight. During a Mir mission a machine that was designed to simulate “heel strike” for use by the astronauts. It was found that the mechanism reduced the amount of bone loss in the calcaneous while under zero gravity conditions but did not prevent it all together. The astronauts used the mechanism as an integral part of the experiment regularly during space flight. The mechanism was incorporated into a strap down system so the astronauts would not be catapulted into the nearest bulkhead. (Goodship et al,1998) Below is an illustration showing the mechanism that illustrates the spring action of the heel strike plate. The foot is strapped into the machine and the spring plate is repeatedly loaded then released striking the calcaneus. (Fig 6)
Exercising has proven to prevent muscle losses but cannot control or alter bone losses. Numerous ideas have been tested to prevent bone loss in space. Of these ideas there have been various animal experiments using growth factors, exercise experiments and vibration or impact tests such as previously mentioned. For space travel to be successful it is imperative for the issues of bone density losses and mechanical property changes to be addressed and corrected.

**Figure 6** Mechanism Used for Heel Strike Generation (Goodship AE et al 1998)
1.1.4 New Theory for Bone Regeneration

Losses of muscle and bone mass are two important physiological changes observed in astronauts after medium and long-term exposure to micro-gravity conditions. An intense exercise program can effectively control muscle mass loss during exposure but there is no effective method so far to control the bone loss. In addition, bone mass recovery in astronauts is a very slow process and it is not clear that the pre-flight bone mineral density is ever recovered. Due to the serious risks of injuries associated with a decreased skeletal strength, bone loss during prolonged space flights may be the most critical obstacle facing space exploration and colonization.

Bone growth and absorption are complicated issues. The mechanical loading environment is believed to play an important role in physiological bone maintenance and remodeling by providing proper signals for bone cell creation and resorption. (Qin et al, 2003). Prolonged unloading associated with micro-gravity alters the dynamics of bone turnover, resulting in the bone loss and skeleton weakness. (6) It has become increasingly evident that a rapid and substantial flow of interstitial fluid occurs across the cortex of bone. Interstitial fluid is driven radially outward across the cortex by the pressure gradient between the blood vasculature (7) and the lymphatic drainage system at the periosteal surface (from the middle of the Haversian system to periosteum in Fig. 1). Localized compression of porous and elastic bone tissue from mechanical loading (body weight due to gravity, action force due to exercise, etc.) also generates transcortical interstitial fluid flow. (3,8,10,12,24) The shear stress and the shear rate induced by this interstitial fluid flow have been shown to mediate cytoskeletal components/membrane...
fluidity and also shown to be mechanical signals mediating production of crucial chemicals controlling the bone metabolisms (see Fig 7 and 8).

1.1.5 Specific Aims and Methods

Weinbaum et al. (1994) found that the signal for bone cell generation by the body weight or action force is transferred to the bone cell (osteoblasts) by the shear stress and shear rate in the canalculus with a fiber filled fluid annulus surrounding osteocytic processes (see Fig 7). We will first adapt their model to calculate the detailed distribution of shear and shear rate in our animal model with and without gravity.

Figure 7 The Cortical Bone. (Weinbaum et al, 1994)

The cortical bone which comprises the shaft of long bones (femur) has an osteonal structure shown above. The cylindrical osteon, is bounded on its outer surface by a cement line and on its inner surface by an Haversian canal, which houses the bone capillaries. The inner surface of the Haversian canal is covered by bone lining cells or osteoblasts which produce new bone. The osteocytes which are embedded in the
mineralized bone matrix have long slender processes (150-200nm diameter and 30-40\(\mu\)m length) which are connected to each other and to the osteoblastic cell processes through gap junctions at their apical tips as shown in Fig 8.

We expect the effect of increased permeability will have similar effect induced by gravity on the shear and shear rate distribution.

The fluid sheath surrounding the membranes of the osteocytes and their processes is contained within a mineralized cavity called the lacunar-canalicular porosity. The canaliculi are the mineralized tubes that surround the osteocytic cell processes. This structure allows for an interconnected communicating network of cells that allows bone tissue to adapt to mechanical strain. Fig. 8. is the enlargement of lacunar-canalicular system in Fig. 7., showing the linking of osteocytes (1) in calcified lamellar bone (3). Two lamellae (2) with
different collagen fiber orientations (7) are visible. Cell processes (5) housed in canaliculi (6) are linked to each other by gap junctions (dark arrows) at their apical tips. Both the osteocytes and their cell processes are surrounded by an unmineralized fluid layer (4).

As shown in the diagram, in Fig 9., as the velocity of interstitial fluid flow increases the drag force along the cell walls increase, thus deforming the cell membrane.

![Cell Membrane Deformation](image)

**Figure 9** Cell Membrane Deformation Caused by Increased Drag (You L. et al, 2001)

For the objective it will be to experimentally test the hypothesis by examining the bone mass and mechanical properties of rat femur for four groups of animals.

a) The control group which is kept in a cage and fed generic diet,

b) Hyperthermia group whose femur exposes to a bio-frequency spectrum light, 2 hours per day. This bio-frequency spectrum light has wave lengths ranging from infrared to micrometer and its energy can induce moderate hyperthermia (local temperature ~
28°C), hyperthermia usually increase microvessel permeability within physiological ranges (Chen et al., 1995); This group is the BFS group.

c) A tail suspended group that is fed a generic diet and will be exposed to BFS. This group is the HBFS group.

d) A tail suspended group merely given a generic diet without the exposure to BFS. This group is the HC group or hanging control group.

1.2 Objectives of This Study

1.2.1 Hypothesis

It is theorized that weakened mechanical properties of bone under microgravity can be minimized if the same shear stresses and shear rates under gravity are achieved by increasing the blood vessel permeability to raise the interstitial fluid flow. (15)

1.2.2 Objective of This Study

The objective of the proposed research is to test the above hypothesis that loss of mechanical properties of bone under micro-gravity can be inhibited if the same shear stress and shear rate distribution under earth gravity (body weight) is achieved by increasing the blood vessel permeability to a level which closely matches the forces generated in a field of gravity. (7)

1.3 Definition of Terms

The following terms are used at various places throughout this thesis:

E = Young’s Modulus which is the ratio of stress over strain or the modulus of elasticity that measures an object’s resistance to deformation.
ε = Strain and is a ratio of the change in length or displacement of a specimen with respect to the original length.

σ = Stress and is the ratio of force over area applied.

I = moment of inertia associated with a particular geometric shape of an object being tested.

P = load applied to an object or pore pressure as it relates to interstitial fluid spaces.

δ = displacement from original position to another point giving deformation to the tested specimen.

k = constant as a ratio of the load vs displacement in the linear region of curve describing the tested results.

SGP = The strain generated streaming potential

GAG = glycosaminoglycans.
CHAPTER 2

EXPERIMENTAL DESIGN AND METHODS

2.1 General Description of Experimental Design

The technique proposed is to experimentally test the hypothesis by examining the bone mass and mechanical properties of rat femur for four groups of animals:

a) The control group which is kept in a cage and fed a generic diet,

b) Another group will be fed a generic diet and to be exposed to a BFS lamp (infrared to micrometer wavelength), which will raise the skin surface temperature to roughly 25-28 °C and increases micro-vessel permeability within physiological ranges. These rats will be kept in a normal cage.

c) A third group with the exposure to a BFS lamp will be suspended via tail suspension and fed a generic diet.

d) Finally, a fourth group will be suspended via tail suspension without BFS and b fed a generic diet.

After a period of 50 days the rats in each group will then be examined for variances in mechanical properties of the bone as it relates to the conditions they were or were not exposed to. The data collected will then be analyzed and translated into a published research paper and final thesis.
2.1.1 Description of Animals Being Tested and Length of Experiment.

All rats were SD, \( \sim 250\text{g} \) initial weight. There were eight rats in each group, half are male and half female. There were four groups of rats; a control group that are simply raised, a second group that were exposed to a bio-frequency spectrum light for 2 hours once a day and house normally as with the control group, a third group that were tail suspended (1-6, 8, 9, 12, 17) and exposed to a BFS lamp for 2 hours once a day, and a fourth that were hind-limb unloaded via tail suspension without BFS exposure. Rats in the four groups were raised for two months and then sacrificed. After sacrificing the animals, fresh femurs and humeri are obtained, cleaned and mass weighed. The mechanical property, Young’s modulus, is measured using a material testing machine in the UNLV Department of Mechanical Engineering. The bone holder in the test machine will be designed and made by the student. Results for different groups are compared to examine the hypothesis.

2.1.2 Bone Extraction

2.1.2 a.) Rat Preparation

After 50 days under various conditions the rats were then euthanized using an overdose of Sodium Pentathol to the heart. Each rat was weighed and femurs and humeri extracted according to the following protocol for later study.

2.1.2 b.) Femur Extraction:

The femur was extracted by first cutting the hide of the rear leg with scissors along the thigh from the knee to the hip. Once the hide is open and muscles are exposed (Figure 13) an incision was then made with a scalpel from the area of the greater trochanter along the facia latae and along side the vastus lateralis and adductor magnus to just below the
patella. The lateral and medial ligaments are then cut from the knee so that the anterior and posterior collateral ligaments could be severed. The muscle tissue of the thigh was then stripped away from the femur upwards toward the hip. The quadratus femoris and obturator externus were then severed as close to the lesser trochanter as possible. The quadratus lumborum, psoas major and iliacus were the next to be severed. This movement opened a gap so that the acetabulum could then be accessed for femoral head removal from the ilium. These muscles are shown in Figs 2.1 below. At this point the femur can then be lifted out of the leg and placed in a vial of formulin, Chaisson (1980)

Figure 10  Musculature of the Hind Limb of a Rat (Chaisson, 1980)
2.1.2 c.) Humeri Extraction

For the humeri the procedure is considerably altered as different muscles are severed and the construction of the forelimb is much different than the hind limb. For the forelimb it was found much easier to sever the muscles from the olcranon process of the elbow up along the lateral side of the biceps and brachialis to the shoulder. At the shoulder it is necessary to first sever the deltoid at point of insertion and then the tendons holding the humerus to the scapula and clavicle which make up the shoulder ball joint. These tendons mentioned include the following: supraspinatus, acromiobraephalus, spinodeltoideus, subscapularis, teres major and minor, and the infraspinatus muscles as seen in Figures 12. and 13.
Figure 12  Lateral View of the Shoulder and Head of a Rat (Chaisson, 1980) (notice the lack of landmarks available to determine the best surgical access to the humeri)

Figure 13  Lateral and Medial Views of the Forelimb of a Rat (Chaisson, 1980)
2.1.3 Bone Preservation

One set of one femur and one humerus was then placed in a vial and labeled according to which rat and from which group it was taken from. The vial was then filled with 10% formulin and placed in the refrigerator for later testing. Once the bones had been harvested and preserved the carcass was then disposed of through the Animal Care Facility at the University of Nevada, Las Vegas.

2.2 Experiment Set-up

2.2.1. Component Construction

For the construction of the track system it is first necessary to use existing tools or construct special tools for the manufacturing of the necessary components. For the wheels of the track mechanism on the side rails a bracket was required that would hold both the axel and a set of two wheels to ride on the rails. Below is a figure of two fitted shaped presses that were used to bend the required bracket. These presses were milled from two pieces of aluminum stock and used to press a piece of 20-gage steel that measured 2.5” in length and 0.75” in width.
Once the brackets were pressed three holes with a diameter of 3/16 in were drilled: two in the bottom side corners and one in the center near the top. The first two were for the wheels to be mounted that would ride on the rails. The remaining hole was where the central axel was mounted for the tail suspension device to ride on. This process is continued for thirty-two brackets and once completed were put aside for later assembly.

Figure 14  Bending Blocks for Suspension Brackets

Figure 15  Suspension Brackets with Axle and Wheels Attached

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
For each rat cage the necessary pulleys are two on each side and a fifth made of steel in the middle to hold up the rat. With twenty cages this would mean one hundred pulleys. The steel will roll more smoothly and can be used for the main pulley suspending the rat. The aluminum is much lighter and easier to manufacture and will be used as the four bracket pulleys. The pulleys were first made using 1” steel for the single center pulley holding the rat directly, then changing to 1” aluminum for four side wheels. The pulleys were made by shaping a cutting tool for proper fit. Once the cutting tool was shaped, the lathe had to be set up for proper cutting angles. The lathe setup requires that the material being lathed is set into place in the rotational grippers. The material must be aligned with the centerline to prevent vibration of the material while being lathed. First a hole was drilled in the end of the 1 in. round stock as in Fig 16.

Once the 3/16” center hole is made, adjustments are made to the tool holder on three dimensions of movement can be made then the cutting tool is utilized to cut the pulley surface. The tool holder is first placed inline with the horizontal or “x” axis of the material
being cut. Once the alignment is made vertical or "y" axis adjustment is then necessary. At this time with the cutting tool clear of the material in the "z" direction, the machine is started and slowly the cutting tool is brought up to the material in the z direction. At approximately 1200 rpm the cutting tool is pressed into the round stock at a rate of 0.015-0.02 of an inch each inward adjustment. The inward movement is made each time that cutting stops. This process continues until the correct diameter of the pulley is acquired. Once the pulleys are finished they are placed in a box for later assembly.

The rails are cut to 17” in length and are 3/8” diameter. The axels are with the same diameter and cut to 8” in length. The number of rails is thirty two with the axels being sixteen. They are put aside for later assembly and the pulley with tail suspension device is then made to ride on the axel. Using the steel pulley and more of the 20 gage flat steel stock the pulley mechanism for suspension is made. A hole of 3/16” in diameter is placed in the bottom of the tail suspension bracket and a fishing swivel is attached to this hole. This swivel allows for the turning around of the rat without injuring its tail. Once the tail suspension devices are made they are placed aside for later assembly.

![Figure 17](image)

**Figure 17** Tail Suspension Clip with Pulley on Axle
The Plexiglas housing is now constructed using ¼” thick Plexiglas. Measuring 17.5” in length and 9.5” in width the housing will fit on the current housing pans used for the rats in the laboratory animal housing facility. Instead of building completely new cages this option was chosen for its cost effectiveness. It was thought best at the time to alter existing cages instead of building complete cages. The Plexiglas housing was ordered from a supplier already cut to the specifications needed. Additional Plexiglas was used to lock the housing in place on top of the housing pans. Once the pieces arrived they were glued using a acrylic bonding agent and assembled with the rails for the tracking mechanism.

![Figure 18 Complete Suspension Cage Mounted on Standard Rat Container](image-url)
2.2.2 Taping the Tail

The taping of the tail is a meticulous process as the tail must be cleaned and clear of all dead skin and soil. First the tails were cleaned with an alcohol and betadine solution. Next a special adhesive named Derma-bond was applied to the skin. Much like super glue this adhesive is very strong and provided the equivalent strength of healed skin of ten days old. Once the Derma-bond became tacky the filament tape strips were applied longitudinally with a paperclip attached. These strips intact both anteriorly and posteriorly precluded tape bands being placed around the tail in two and sometime three places depending on the weight of the rat. The final taped tail was then attached to the fishing swivel that was connected to the axel pulley. The illustration below depicts the placement of tapes and clips.

![Diagram of tail taping process](image)

**Figure 19** Tail Taping Detail (Morey-Holton et al 2001)
Extreme care was taken not to apply the tape too tight or too loose. If the tape was too tight, necropsy of the tail would occur and the rat would suffer undo stress. If the tape was too loose the rat would eventually slip out of the tape and no longer be suspended. The picture on the next page depicts how the rat is actually suspended via a tail restraining device.

Illustration 1  View of Rat Suspended from Rear
Below are the cages of the control group without BFS exposure and those that are not suspended but are being exposed to the BFS lamp. Each of these cages had to be cleaned and new food and water placed daily. The rats being exposed to BFS were taken down.
Monday through Friday for a period of two hours at six cages at a time for a total of four shifts.

Illustration 3 Cage Storage of Control and BFS Groups

The picture below shows how the cages were placed under the large BFS lamp, while two more cages had much smaller lamps placed above each individually.
2.2.3 Biofrequency Spectrum Lamp

The BFS lamp is a low frequency light wave lamp in the infrared to microwave range and is the term used for both the frequency and spectrum of physical data and parameters being projected from a living organism, Chen et al (1995). The graph on the following page depicts the band or shaded region between two wavelengths that the BFS operates.
Living organisms radiate energy to the surrounding space at varying degrees based on health and the organism's functional status based on the physical information emitted and its correlation. The energy levels emitted are not strong but are significant enough to use as the basis of biofeedback. The BFS was developed to simulate these energy levels and send it back to the living organism. (Chen et al, 1995). The key points to the BFS lamp theory is as follows:

- The living organism emits low levels of energy that can appear as many kinds of physical signals ranging from infrared to weak microwave. The combination of these parameters, and the frequency and spectrum make up the BFS.
- The BFS lamp was designed on the basis of bionic theory that can generate and emit a frequency and spectrum of energy closely resembling the living organism that it was designed after.
- The maximum energy absorption will occur when there is frequency of resonance with the living organism.
In this particular study the BFS will be used on rats and will be used to maintain an estimated temperature of 25-28 degrees Celsius at an estimated 40 cm from the surface of the skin. The rats in this particular group will be monitored closely to prevent hyperthermia.

2.2.4 Tail Suspension Technique

The following are the design parameters for the tail suspension method as part of this experiment, (Morey-Holton, 2001):

- The maximum head tilt angle is 30 degrees.
- The length of the rat is roughly 15 inches.
- The back feet need to be off the floor of the cage. This makes a 12 inch distance from the floor to the tip of a raised tail.
- The rat needs to be able to freely move about the cage.

![Tail Suspension Method](image)

**Figure 20** Design Parameters for Tail Suspension
With these parameters kept in mind the design described in the next few pages was achieved for the rail system that would allow free movement of the rat while maintaining a 30 degree head tilt with hind legs lifted. The use of rails, pulleys and axels are used to provide two-dimensional movement of the rat. Ideally the rats will not climb up their own tails for relieving pressure on their tail. The rails with pulleys attached to axels will allow for movement in the X direction while the axels supporting an individual pulley will provide movement in the Y direction. The following illustrations show the method by which the rats were suspended.

Illustration 5 View of Suspended Rat from the Side
2.2.5 Test Source Machine for “E” (young's Modulus) Test

It is hoped that the method of suspension with BFS will show that mechanical property weaknesses of the trabecular and compact areas would be minimized. The bone from the rats were tested for strength by using a Test Resources Q100 strength testing machine during three point bending.

The tensile means of testing fresh rat bone repeatedly proved to be very difficult as the holders would slip when producing a very erratic stress vs. strain curve or using
binding methods would produce a two part curve. The binding method used was a nylon grommet around the diaphysis of the bone. As more force in the tensile direction was applied the grommet would tighten circumferentially around the bone and no slip would occur. The problem with this method is the inclusion of the nylon rope's own stress factors and the inability to determine accurately the stress vs. strain for the rat bone. After many tests it was decided to test the bones using a three point bending method as in a study by (Garger et al, 2000). With this method it is necessary to track the load vs. displacement and then calculate a constant factor so that Young's Modulus could then be determined for the rat bone. The final setup of the Q100 was in compression with a hollowed out cylinder for the use and containment for the three point bending application of the bones and is illustrated below.

Illustration 7 Q100 with Humerous in Compression for 3 Point Bending
2.2.4 a.) Test Resources Q100 Testing Machine

The Q100 testing machine was purchased and used to test for bone strength. The machine was chosen for its close tolerances and ability to test at very slow speeds and small force increments. The process uses a transducer that senses the amount of force applied which is then linked to a computer on the upper end of the force-loading stand. On the lower end of the stand is a computer controlled force application motor. The Q100 sends the data to the computer after the maximum set load is obtained. The break of the specimen also sends the information to the computer where it is automatically opened up in a Microsoft Excel spread sheet. For this experiment the rate that was determined to test the bones, by means of extensive tests on bones from another experiment. The test speed
was set at 0.01 mm/sec and the maximum loading was set at a high enough level to allow completion of the test. At this rate there was sufficient time to collect enough data points as to make a smooth and distinct load vs. displacement curve.

2.3 Young's Modulus Measurement

The following picture shows that the displacement (δ) is the distance the deflection of the horizontal line of application is being moved in the downward direction. L is the distance between horizontal supports the ends of the bone are being supported by, with the force being applied at the half way point through L.

\[
\frac{d^2\delta}{dx^2} = \frac{-M}{EI}
\]

where \( \delta \) is the displacement, \( x \) is the overall length, \( E \) is Young's Modulus and \( M \) is the moment.

**Figure 21** Force Application Over a 3 Point Bending Device. (What is seen here with the hashed marks is the large steel item seen in the previous illustrations.)
Through integration and substitution of load times moment arm for M the following equation is attained:

\[ E I \frac{d^2 \delta}{dx^2} = -W \frac{x^2}{4} + C_1 \]  

at \( x = L/2 \), \( d\delta/dx = 0 \). Therefore \( C_1 = WL^2/16 \)

By integration once more the equation then becomes this result on the following page:

\[ E I \delta_{\text{max}} = -W \frac{x^3}{12} + W \frac{L^2 x}{16} + C_2 \]  

at \( x = 0 \), \( \delta = 0 \), then \( C_2 = 0 \) so \( \delta \) is max at \( x = 0 \)

so

\[ E I \delta_{\text{max}} = -W \frac{(L/2)^3}{12} + W \frac{L^2 L}{16} = -W \frac{(L/8)^3}{12} + W \frac{L^3}{32} = -W \frac{L^3}{96} + W \frac{L^3}{32} \]

The final equation is then determined to be

\[ E = \frac{W \cdot \frac{L^3}{48 \cdot I \cdot \delta_{\text{max}}}} \]

Where \( W \) is constant and can be described as \( k \) which is also the slope of the line in the linear region of the load vs. displacement curve described in graphs 3.1 b.) and 3.2 b.) and is equal to \( W/\delta \)

\[ E = kL^3/48 I \]

The bones being studied were treated as of cylindrical shapes for ease of calculations.

To determine the Young's Modulus first the moment of inertia had to be calculated and is obtained by using the following equation:
The radii are calculated using the outside circumference, average of two inside diameters based on an ellipse and then converted into the outside and inside radii, respectively, of a circle as depicted in the drawing bin figure 22.

\[ I = \frac{1}{4} \pi \left( r_0^4 - r_1^4 \right) \]

**Figure 22** Radii Averaging

Once the moment of inertia is calculated the next step was to determine a constant that shows the relationship of load vs. displacement in the linear region of the tested bones. This constant is the slope of the line in the linear region of the load vs. displacement curve. The constant is then used with the moment of inertia, the spread or length of bone a load is being applied and the speed at which the load is being applied to determine the Young’s Modulus. The following equation is then used to determine the Young’s Modulus for the bones studied:

\[ E = kL^3/48 \ I \]
CHAPTER 3

EXPERIMENTAL RESULTS

The final results of this project illustrates the use of BFS as a possible means for retarding the effects of micro-gravity or zero gravity conditions on the mechanical properties of bone. Through careful analysis of the data those rats with BFS exposure had a generally higher level stress/strain ratio or Young’s Modulus than those rats without the exposure.

3.1 Data for Femur

For the control group the following load vs. displacement curves exhibiting the data for a loaded femur are shown below and following page.

Graph 2 Load vs. Displacement for a Femur
Graph 3  Linear Region of Load vs Displacement for Humerus (k constant = 222.79)

This value is used to determine the Young’s Modulus based the equation using displacement instead of stress vs. strain.

Graph 4  Young’s Modulus of Femurs
The previous graph shows the results for the femurs studied. There is a similar increase between the control group and that of BFS as compared to the difference with the HC and HBFS group. One can see the drop of YM between the control and the HC as is expected due to the loss of weight bearing on the hind limbs. The increase in YM with the BFS and HBFS groups from their counterparts can possibly be contributed to the exposure to BFS waves.

The graph shows that for the femurs studied there is an increase in Young’s Modulus (YM) from the Control to the BFS exposed, non-suspended (BFS) groups and there is also an increase in the YM from the suspended, non-BFS (HC) to the BFS exposed and suspended (HBFS) group. The increases range from 1-1.2% between the two comparative pairs. The average strain rate in this study is at 9.2367E-05 with an average YM for the each group being i) 1.78 GPa, ii) 2.04 GPa, iii) 2.03 GPa, and iv) 2.04 GPa;

Below is the statistical analysis completed on the data for the femurs. The analysis shows that a non-significant change occurred between the control groups and those exposed to the BFS lamp.

<table>
<thead>
<tr>
<th>Table 1. t-Test: Two-Sample Assuming Equal Variances</th>
<th>Control vs BFS Normal Caged (femur)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable 1</td>
<td>Variable 2</td>
</tr>
<tr>
<td>Mean</td>
<td>1.774155929</td>
</tr>
<tr>
<td>Variance</td>
<td>0.257241882</td>
</tr>
<tr>
<td>Observations</td>
<td>14</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>0.346687168</td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0</td>
</tr>
<tr>
<td>Df</td>
<td>25</td>
</tr>
<tr>
<td>t Stat</td>
<td>-0.798831453</td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.215956125</td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.708140189</td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.431912251</td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.05953711</td>
</tr>
</tbody>
</table>
Table 2. t-Test: Assuming Equal Variances  Suspended Control vs. Suspended BFS for femur

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable 1</th>
<th>Variable 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.42677225</td>
<td>2.027379775</td>
</tr>
<tr>
<td>Variance</td>
<td>2.590328723</td>
<td>0.411595893</td>
</tr>
<tr>
<td>Observations</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>1.500962308</td>
<td>12</td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Df</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>t Stat</td>
<td>0.798528847</td>
<td>0.798528847</td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.216549956</td>
<td>0.216549956</td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.717144187</td>
<td>1.717144187</td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.433099912</td>
<td>0.433099912</td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.073875294</td>
<td>2.073875294</td>
</tr>
</tbody>
</table>

Based on the tables of probability vs degrees of freedom for a biological work the calculated “t” value is less than the value given in the table, thus the test is non-significant in it’s findings.

3.2 Data for Humerus

For the control group the following load vs. displacement and stress vs. strain curves exhibiting the data for the loaded humeri are shown.

Graph 5 Load vs. Displacement for Humerus
Graph 6  Linear Region of Load vs Displacement for Humerus (k constant =125.4)

Once the slope of the linear portion is found, it is used as the constant in calculating the Young’s Modulus as explained in a previous equation in the Test Resources Q100 section.

Graph 7  Young’s Modulus for Humeri
Based on the graph 3.2 d.) one can see that there is an approximate difference of 12% rise in Young's modulus with the exposure to BFS light. This can be attributed to a possible connection to those rats with BFS lamp technology and that of those without the BFS lamp. The graph shows that for the humeri studied there is an increase in Young’s Modulus (YM) between the Control and the BFS exposed, non-suspended (BFS) groups and there is also an increase in the YM from the suspended, non-BFS (HC) and the BFS exposed and suspended (HBFS) group. The increases range from 10-15% between the two comparative pairs. The average strain rate in this study is at 8.6977E-05 with an average YM for each group of rat humeri are i) 3.83 GPa, ii) 4.14 GPa, iii) 3.78GPa, and iv) 3.80 GPa. The error rate for these groups are 0.33, 0.31, 0.41, and 0.31 respectively.

Below is the statistical analysis completed on the data for the femurs. The analysis shows that a non-significant change occurred between the control groups and those exposed to the BFS lamp.

**Table 3. t-Test: Two-Sample Assuming Equal Variances**

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.812952</td>
</tr>
<tr>
<td>Variance</td>
<td>1.344725</td>
</tr>
<tr>
<td>Observations</td>
<td>11</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>1.229555</td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0</td>
</tr>
<tr>
<td>Df</td>
<td>21</td>
</tr>
<tr>
<td>t Stat</td>
<td>-0.7036</td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.244707</td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.720744</td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.489413</td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.079614</td>
</tr>
</tbody>
</table>
Table 4. t-Test: Two-Sample Assuming Equal Variances Suspended Control vs. Suspended BFS for Humeri

<table>
<thead>
<tr>
<th></th>
<th>Variable 1</th>
<th>Variable 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.056248</td>
<td>3.802888</td>
</tr>
<tr>
<td>Variance</td>
<td>3.777716</td>
<td>1.169491</td>
</tr>
<tr>
<td>Observations</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>2.473604</td>
<td></td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Df</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>t Stat</td>
<td>0.394591</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.348472</td>
<td></td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.717144</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.696944</td>
<td></td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.073875</td>
<td></td>
</tr>
</tbody>
</table>

Based on the table of probability vs degrees of freedom for a biological work the calculated “t” value is less than the value given in the table, thus the test is non-significant in its findings.

3.3 Average Comparison of Bones Being Studied

By comparing the average load vs. displacement of each of the four groups of both the femur and humeri the following chart shows the differing trends of the subsequent groups. The loads in the groups exposed to BFS are slightly higher. In the case of the hanging control vs. the hanging with BFS the displacement is higher with an applied load before breakage. In the case of the control group vs. BFS (non-suspended) the BFS group shows that the force required for breaking the bones is higher in the final stages of breakage. For the HC vs. HBFS the HBFS attains higher displacement with the same force applied.
Graph 8 Averaged Load vs. Displacement for 4 Groups

Using the standard error equation below, the data was analyzed and the subsequent bar graph was attained showing the differences in Young’s Modulus amongst the four different groups.

\[ s = \text{series number} \]

\[ i = \text{point number in series } s \]

\[ m = \text{number of series for point } y \text{ in chart} \]

\[ n = \text{number of points in each series} \]

\[ y_{is} = \text{data value of series } s \text{ and the } i^{th} \text{ point} \]

\[ n_y = \text{total number of data values in all series} \]

\[
\text{SE} = \frac{\sum_{s=1}^{m} \sum_{i=1}^{n} y_{is}^2}{(n_y - 1) \cdot (n_y)}
\]
CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 Problems and Error Analysis

This experiment covered the application of BFS to rats in a tail suspended application. The purpose for tail suspension is clear in that the simulation of micro gravity conditions can be met. This experiment showed that the application of BFS lamps can reduce the losses of mechanical properties in bone under micro gravity conditions. These results show exposure to BFS can be applied to the reduction of bone density losses experienced in bed ridden patients as well as those suffering from osteoporosis. Although it is not perfectly clear how BFS can help all bone loss reduction cases it does show that it possess the potential to help reduce the negative responses of bone to the micro gravity. This experiment was posed with various problems as it pertains to the successful application of tail suspension of rats. In the following subsection these problems are addressed.

4.1.1 Problems Incurred During Experiment

One of the problems experienced with the rats in this experiment was that some rats developed ways to reload their back legs during the suspension and eventually had to be removed from the suspension portion of the experiment for non-compliance. A picture of this occurrence is shown in Illus. 4.1 on the following page. The collar device was an
attempt to keep the rats from either chewing on their tail or attempting to climb its own tail as this one below still managed to do.

Illustration 9 Tethered Rat Trying to Escape

Another problem that plagued the experiment is the necropsy of the tails of some of the rats. This was an occurrence later on in the experiment and should be considered before replicating or another study involving tail suspension.
Illustration 10  Necrotic Tail (notice the black dead end of the rat tail. This discoloration is evident of dead tissue caused by the loss of circulation.)

The most notable problem among the rats was the lack of traction within the altered cages. The cages used did not allow for the rats to grasp the bottom of the cage to pull themselves along. This ability would have prevented many of the rats from climbing their tails, chew the tape or even chew their own tail in an attempt to get free. In Illus. 11. is a picture of something we discovered at the end of the experiment to compensate for the loss of traction.
Illustration 11 This Illustrates the Use of a Rubber Matting

On the bottom of the cage rubber matting was placed to allow the rat to pull themselves around the cage with forearms. Notice the collar device, this was used to prevent the rat from chewing its own tail off in an attempt to free themselves.
A problem was also found with humeral head after placing in solution… the head separates at the growth plate while cleaning soft tissue off the bone. This does not interfere with testing however it does make measuring bone density difficult. The reason for the dislocation of the head is that the rats had not completely reached maturity when sacrificed and the growth plate had not yet solidified. The tension method of testing becomes nearly impossible due to the non-solidification of the growth plate of the bone.

4.1.2 Corrective Measures for Future Tail Suspension Experiments

To prevent the rats from climbing up their tails and to prevent the rats from pressing against the sides of the cage the following design should imperatively be used. This design of a rat cage for tail suspension is based on giving the rat something to grip for mobility, waste management and reduced stress on the rat. This experiment was attempted to replicate the tail suspension device for this design at the same time attempting to minimize cost. As a result the best design seen in figure 4.1 is expressed in Emily Morey-Holton’s description and should be followed explicitly for all tail suspension experiments.
Figure 23 Morey-Holton Design for Tail Suspension Cages, Morey-Holton (2002)

4.2 Conclusion

The experiment shows that the application of BFS can improve the effects of blood vessel permeability on the mechanical properties of bone in micro or zero gravity. The effects are that the Young’s Modulus (YM) is increased as it relates to the coordinating control group. The BFS group has a higher averaged YM than the control group and the suspended BFS group has a higher averaged YM than that of the suspended control group. The overall implications can possibly include a means of improving the bone
density and strength of bone in bed ridden patients as well as those suffering from osteoporosis or other bone related diseases.

4.3 Future Study

For future studies with this project there will be further investigation into the histological aspects of bone development under micro-gravity conditions. These studies will show the cellular growth of the bone under the controlled conditions of the development of these rats. During the last ten days of raising these rats an injection of Calcein was given to all rats at ten and three days before sacrificing. The Calcein, colored much like iodine, is absorbed by the bone and becomes a marker for bone growth. Separating the injections allow for a change in bone growth to be observed and evaluated. The Calcein was administered at 1 % of each rat’s body weight per injection. Once the bones were harvested they were kept in 10 % formulin and refrigerated for later study. After the initial mechanical loading tests were completed the broken bones are placed back into the formulin and will be used for histological evaluation for growth and remodeling.
REFERENCES


3. Bikle DD, Halloran BP; The response of bone to unloading: J. Bone Miner Metab. 1999; Vol. 17; pp. 233-244


7. Bronk JT, Meadows TH, Kelly PJ; The relationship of increased capillary filtration and bone formation; Clinical Orthopaedics and Related Research, No. 293, pp 338-345 1993


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
22. Sibonga JD, Zhang M, Evans GL, Westerlind KC, Cavolina JM, Morey-Holton E, Turner RT; Effects of Spaceflight and Simulated Weightlessness on Longitudinal Bone Growth; Bone October 2000; Vol. 27, No. 4; pp. 535-540


VITA

Graduate College
University of Nevada at Las Vegas
Robert L. Drollinger

Home Address:
1405 Vegas Valley Drive #260
Las Vegas, NV, 89109

Degree:
Bachelor of Science, Mechanical Engineering with minor in Biology, 2000
University of Nevada at Las Vegas, Las Vegas, NV, USA

Special Honors and Awards:
1999 NSF Summer Fellowship recipient
Student member of Biomedical Engineering Society

Poster Presentations:
1. Analysis for Pressure Sensation in Prosthetic Socket for Lower Extremity Amputees Symposium for Orthotists and Prosthetists in San Diego 2001

Thesis Examination Committee
Chairperson, Dr. Bingmei Fu, Ph.D.
Committee Member, Dr. William Culbreth, Ph.D.
Committee Member, Dr. Edward Neumann, Ph.D.
Graduate Faculty Representative, Dr. John Mercer, Ph.D.