Brigham Young University
BYU ScholarsArchive

All Theses and Dissertations

2010-07-09

# The Effects of Low Frequency Ultrasound in Transdermal Drug Delivery

Aaron M. Wells
*Brigham Young University - Provo*

Follow this and additional works at: https://scholarsarchive.byu.edu/etd

 Part of the Exercise Science Commons

BYU ScholarsArchive Citation

Wells, Aaron M., "The Effects of Low Frequency Ultrasound in Transdermal Drug Delivery" (2010). *All Theses and Dissertations*. 2560.
https://scholarsarchive.byu.edu/etd/2560

This Dissertation is brought to you for free and open access by BYU ScholarsArchive. It has been accepted for inclusion in All Theses and Dissertations by an authorized administrator of BYU ScholarsArchive. For more information, please contact scholarsarchive@byu.edu, ellen_amatangelo@byu.edu.

# The Effects Of Low Frequency Ultrasound

# In Transdermal Drug Delivery

Aaron M. Wells

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

David O. Draper, Chair
Kenneth L. Knight
Gary W. Mack
Paul J. Fields
William G. Pitt

Department of Exercise Sciences

Brigham Young University

August 2010

Copyright © 2010 Aaron M. Wells
All Rights Reserved

ABSTRACT

The Effects Of Low Frequency Ultrasound

In Transdermal Drug Delivery

Aaron M. Wells

Department of Exercise Sciences

Doctor of Philosophy

*Objective:* Determine if varying ultrasound frequency affects the delivery of 10% hydrocortisone concentrations during phonophoresis. Utilize intramuscular microdialysis probe for drug collection, thus improving the experimental model. *Methods:* Thirty one (10 in groups 1 and 2, 11 in group 3) healthy subjects participated in this study. *Interventions:* Subjects were randomly assigned to one of three treatment groups receiving 10 minute ultrasound treatments applied to a standardized area of the gastrocnemius muscle of the right leg. The ultrasound was performed over the treated area using a 10% hydrocortisone compound mixed with standard ultrasound gel. The contralateral limb served as the control (no mixed compound or treatment) for all groups. Group one received sham ultrasound. Medicated gel was placed on the treatment site, the sound head moved, but no ultrasound was applied. Group two received 45 KHz at .056 $w/cm^2$. Group three received 1 MHz at 1.0 $w/cm^2$ at a 50 % duty cycle. *Results:* There was no difference in cortisol concentration change during treatment between the three treatment groups on the treated limbs (sham = 1.1 ± 7.5 ng/ml, 45 KHz = 1.1 ± 1.5 ng/ml, 1 MHz = 4.1 ± 7.8 ng/ml; $F_{2,22}$ = .34, P = .72) or control limbs (sham = 1.65 ± 6.6 ng/ml, 45 KHz = -1.3 ± 2.7 ng/ml, 1 MHz = 0.37 ± 8.1 ng/ml; $F_{2,22}$ = .67, P = .546). No difference was found in cortisol concentration change during treatment between the treatment limbs and the control limbs (treatment = 2.1 ± 6.2 ng/ml, control = 0.20 ± 5.9 ng/ml; $F_{1,22}$ = .9, P = .35). The following factors were found to influence cortisol concentrations levels in dialysate collected during treatment: depth of muscle in the treatment limbs ($F_{1,22}$ = 6.4, P = .02), microdialysis probe depth in the control limbs ($F_{1,22}$ = 4.1, P = .05), and pre treatment cortisol level in the control limbs ($F_{1,22}$ = 10.1, P = .004. *Conclusions:* There was no evidence altering ultrasound frequency from 45 KHz to 1 MHZ enhanced the delivery of 10% hydrocortisone to treatment tissues under these experimental conditions.

Keywords: microdialysis, 10% hydrocortisone

# ACKNOWLEDGMENTS

Thank you to my entire committee for their direction, assistance, patience and time. You have helped me grow academically, personally, and spiritually. Despite my many limitations, each member has shown me what it means to be a scientist, researcher, and scholar. Dr. Draper, for many years you have believed in me and have given me constant support while also keeping me headed in the right direction. Thanks to our interaction I believe I have a better understanding of what it truly means to be an educator.

Thank you to the many other students, both graduate and undergraduate, whom I have encountered during my time at Brigham Young University. You have been great examples, friends, and tutors. I have learned much from you and am honored to be your colleague.

Lastly and most importantly I would like to thank my family for their love and support. I hope my parents understand how much I appreciate their example and continued love and support. My wife Tiffani continues to encourage and support me through good and bad. This would not have been possible without her. I hope my children can learn and continue to grow, far surpassing what I accomplish.

Table of Contents

List of Tables

List of Figures

Figures Page

# The effects of low frequency ultrasound in transdermal drug delivery

Aaron M. Wells, Ph.D., LAT, ATC [*]
David O. Draper, Ed.D., LAT, ATC[*]
Kenneth L. Knight, Ph.D., LAT, ATC, FACSM[*]
Gary W. Mack, Ph.D.[*]
William G. Pitt, Ph.D.
Paul J. Fields, Ph.D.[†]

[*]Human Performance Research Center, Therapeutic Modality Laboratory, Brigham Young University, Provo, UT

[†]Department of Statistics, Brigham Young University, Provo, UT

This study was partially funded through an internal grant from the Mary Lou Fulton Chair for Health and Human Performance.

Address correspondence to:
Aaron M. Wells Ph.D., ATC
Assistant Professor
106 Smith Field House
Department of Exercise Science
Provo, UT 84602
Email: aaron_wells@byu.edu

**Abstract**

*Objective:* Determine if varying ultrasound frequency affects the delivery of 10% hydrocortisone concentrations during phonophoresis. Utilize intramuscular microdialysis probe for drug collection, thus improving the experimental model. *Methods:* Thirty one (10 in groups 1 and 2, 11 in group 3) healthy subjects participated in this study. *Interventions:* Subjects were randomly assigned to one of three treatment groups receiving 10 minute ultrasound treatments applied to a standardized area of the gastrocnemius muscle of the right leg. The ultrasound was performed over the treated area using a 10% hydrocortisone compound mixed with standard ultrasound gel. The contralateral limb served as the control (no mixed compound or treatment) for all groups. Group one received sham ultrasound. Medicated gel was placed on the treatment site, the sound head moved, but no ultrasound was applied. Group two received 45 KHz at .056 $w/cm^2$. Group three received 1 MHz at 1.0 $w/cm^2$ at a 50 % duty cycle. *Results:* There was no difference in cortisol concentration change during treatment between the three treatment groups on the treated limbs (sham = 1.1 ± 7.5 ng/ml, 45 KHz = 1.1 ± 1.5 ng/ml, 1 MHz = 4.1 ± 7.8 ng/ml; $F_{2,22} = .34$, P = .72) or control limbs (sham = 1.65 ± 6.6 ng/ml, 45 KHz = -1.3 ± 2.7 ng/ml, 1 MHz = 0.37 ± 8.1 ng/ml; $F_{2,22} = .67$, P = .546). No difference was found in cortisol concentration change during treatment between the treatment limbs and the control limbs (treatment = 2.1 ± 6.2 ng/ml, control = 0.20 ± 5.9 ng/ml; $F_{1,22} = .9$, P = .35). The following factors were found to influence cortisol concentrations levels in dialysate collected during treatment: depth of muscle in the treatment limbs ($F_{1,22} = 6.4$, P = .02), microdialysis probe depth in the control limbs ($F_{1,22} = 4.1$, P = .05), and pre treatment cortisol level in the control limbs ($F_{1,22} = 10.1$, P = .004. *Conclusions:* There was no evidence altering ultrasound frequency from

45 KHz to 1 MHZ enhanced the delivery of 10% hydrocortisone to treatment tissues under these experimental conditions.

Keywords:  microdialysis, 10% hydrocortisone

**Introduction**

Medication is commonly used in treating musculoskeletal injury.[1, 2] There are many concerns however, regarding effective and appropriate dosage delivery methods.[2-4] Long term use of some orally ingested medicines can result in damage to the liver, kidneys, and stomach.[3-7] Injected medication can result in excessive patient discomfort, infections, nerve damage, and scar tissue in repeat injection sites.[6] Questions arise as to the dosage required to ensure adequate treatment following first pass through the digestive system, and in the amount of actual drug delivery reaching the target tissues.[1,3, 8-10] These concerns and rising costs in developing new drugs, have contributed to the exploration of alternative methods of drug delivery. Transdermal drug delivery is one such method.

Transdermal drug delivery (TDD) is the administering of medication through the intact skin. It is designed to facilitate more efficient delivery while decreasing many of the complications that arise with traditional drug therapy.[1, 3] Examples of TDD techniques include iontophoresis, medicated skin patches, topical ointments, and phonophoresis, the use of ultrasound waves to facilitate drug delivery.

The therapeutic mechanisms of ultrasound are classed in two categories: thermal and mechanical. The thermal effects and benefits of ultrasound are well documented.[4-6] However, the mechanical or non-thermal effects have a much greater influence on TDD.[11-13] It is these cellular level mechanical phenomena that result in increased skin permeability, thus permitting greater drug delivery to desired tissues.

Unfortunately, despite frequent use of phonophoresis, the literature is inconclusive regarding its effectiveness.[9, 14-20] Much of this disparity is due to two factors: first, questions

regarding appropriate application parameters (i.e., treatment duration, duty cycle, and wave frequency) and second, the lack of an objective method to analyze actual drug delivery.[19, 21-32]

The following research questions directed this study: Does altering ultrasonic frequency enhance the delivery of cortisone (using 10% hydrocortisone cream)? Do pre-treatment cortisol levels, depth to the treated muscle, and depth of the microdialysis collection probes influence the analysis determining if drug delivery occurred?

## Methods

### *Subjects*

Thirty-one healthy college students were recruited. Subjects were both male and female with no preference given to gender. Due to excessive variations of cortisol levels that occur during menstruation, female participation was limited to those currently in the first seven days of the menstrual cycle. Prior to inclusion, each subject completed an information and health and history questionnaire. Persons experiencing a lower extremity injury or general illness within two weeks of their participation in this study, or who had a history of decreased sensation within the last year to the lower legs were excluded.

### *Study Design*

A 2 X 2 X 3 mixed factor design with repeated pre and post measurements guided data collection. Independent variables were time (pre and post treatment), treatment (treated and control), and ultrasound level (sham, 45 KHz, and 1 MHz). The dependant variable was dialysate cortisol level. In addition, the depth of the microdialysis probe (PD) and the depth of the treated muscle (MD) were measured prior to the experiment with Doppler imaging ultrasound.

Drug delivery was determined by measuring cortisol levels in dialysate collected from interstitial fluid prior to and following treatment. Subjects were assigned to one of three

treatment groups. The right leg always received the treatment while the left leg served as a control (no hydrocortisone cream or treatment).

*Instruments*

*Ultrasound Devices and Medicated Cream.* The 1 MHz ultrasound and sham ultrasound applications were applied with an Omnisound 3000 E Ultrasound device (Accelerated Care Plus, Reno, NV) with a 7.2 cm diameter sound head with 5 $cm^2$ crystal at an intensity of 1.0 $W/cm^2$ with a 50% duty cycle to equate treatment of acute injury. The 45 kHz ultrasound treatment was applied with a Duo Son ultrasound device (S.R.A. Developments Ltd, South Devon, United Kingdom) at an intensity of .058 $W/cm^2$ per machine pre-set intensity.

Aquasonic 100 water soluble hypoallergenic ultrasound transmission gel (Parker Laboratories, Inc, Fairfield, NJ) was mixed with hydrocortisone to create a 10% hydrocortisone cream. This cream was prepared by the University Student Health Center Pharmacy.

*Ultrasound Imaging Device.* A Titan Ultrasound System (SonoSite, Bothell, WA) was used to measure treated muscle depth and microdialysis probe depth following insertion.

*Intramuscular Microdialysis Probe*

*Probe Construction.* The microdialysis probe was custom made of static, non-moving components that illicit no reaction to human tissue. Production of the probes was performed under a microscope to ensure proper production and structural integrity. A 37 cm length of spring tempered stainless steel wire (0.002 inch diameter, Alan Baird Ind., NJ) supported the probe during construction and facilitated insertion into the treatment site. The probe consisted of an 8 cm piece of polyimide tubing, 4 cm of hollow fiber dialysis tubing, 4 cm of polyimide tubing, 20 cm of PE 10 tubing, and 6 cm of PE 50 tubing (Figure 1). The 8 cm piece polyimide tubing (0.0064 inch OD, Cole-Parmer, IL) was inserted 1 cm into the hollow fiber tubing and

secured with standard super glue on each end leaving 2 cm of exposed membrane. The completed probe was checked for proper production by passing sterile saline solution through the device to make certain no joints or portions of the probe leaked. Following the testing, probes were packaged individually and gas sterilized with ethylene oxide.

*Probe Recovery Analysis.* Invitro recovery property of the probes was determined by calculating the extraction fraction. To obtain that value, the probe, including the portion containing the hollow fiber, was placed in a shallow tub and bathed in 2 ml of 0.9% saline. The bath was thoroughly mixed while one end of the probe collected samples and the other was attached to the infuser. The probe was perfused at 5.0 µl/min with 0.9% saline. After an equilibration period of 60 minutes, a known quantity of cortisol was added to the bath. Dialysate samples of 100 µl were collected 8 times (every 20 minutes for 160 minutes) to establish a steady state recovery rate for microdialysis probes. The extraction fraction was calculated by dividing the cortisol concentration in the dialysate by the cortisol concentration in the bath. The invitro recovery rate of the microdialysis probes was 33 ± 3 % of cortisol added to the solution.

*Tissue Perfusion.* Probes were perfused using a Harvard Apparatus PHD 2000 Programmable Infusion Pump (Harvard Apparatus, Holliston, MA).

*Drug Delivery Analysis.* The amount of cortisol in dialysate collected from the intramuscular microdialysis probe was measured with a cortisol enzyme immunoassay (Immuno Biological Laboratories, Hamburg, Germany, ninety-six well microtiter plate). Cortisol levels are reported in ng/ml.

*Testing Procedures*

Subjects were placed in a prone position. The treatment and control sites, located in the medial

gastrocnemius proximal to the Achilles tendon on the left and right limb, were cleansed and sterilized.

*Probe Placement.* Using a 27-gauge needle used as a guide cannula, probes were inserted horizontally into the medial gastrocnemius muscle approximately 2 inches superior to the musculotendinous junction of the Achilles tendon in both the treated and control limb.

The probe entrance and exit sites on the skin were at least 6.0 centimeters apart. Cannula depth, muscle penetration and depth of the treated muscle were verified and recorded (cm) at both the treatment and control sites with Doppler ultrasound imaging. After confirming the cannula had penetrated the muscle at both the treatment and control sites, the microdialysis probes were fed through the guide cannula. Following insertion of the probe, the cannula was removed with the probes left in place. Small vials were secured with tape at the ends of the microdialysis probes near the exit sites to collect dialysate.

The probes were perfused with 0.9% sterile saline at a rate of 10µl/min with a Harvard infusion pump for 70 minutes. This allowed tissue to recover from needle and probe insertion. During the first five minutes, the volume of dialysate (fluid coming out of the probe) was monitored. If this volume was significantly lower than expected (indicating a leak) the probe was replaced. At minute 70 of the recovery period the infusion rate was altered to 5µl/min and remained constant for the remainder of the experiment. Dialysate was then collected for 20 minutes to determine pre-treatment cortisol levels. After 20 minutes of collecting dialysate, the vials were replaced. The vials with dialysate were labeled and stored in a freezer for future analysis.

*Drug Delivery Treatment.* We then placed a pre fabricated template to confine the treatment area and ensure consistent treatment size, an area 2 times the size of the 5 cm

ultrasound head (a circle with a 5 cm radius). We then placed the prepared 10 % hydrocortisone coupling gel on the treatment site of the treated limb (Figure 2).

The ultrasound head was moved in circular pattern for the duration of the 10-minute treatment at a rate of approximately 4 cm per second. During the treatment, dialysate from the intramuscular microdialysis probe was collected from both the treatment and control sites for analysis.

At the conclusion of the 10 minute treatment, the medicated cream was carefully removed. Subjects remained in the prone position for an additional 10 minutes while dialysate collection continued to ensure that medication delivered during the ultrasound treatment had passed to the vials. After 10 minutes the saline perfusion was terminated and the collection vials were removed, labeled, and stored in a freezer for later analysis. Then we removed the ultrasound template and the intramuscular microdialysis probes. The portal sites were treated with triple antibiotic and covered with a band aid. Subjects were given a basic wound care guide with contact information should any questions arise.

*Statistical Analysis*

We used a General Linear Model incorporating analysis of covariance and repeated measures to determine if cortisol change was different between the groups. A 2 X 3 ANCOVA with groups (control and treated) and treatment (sham, 45 KHz, 1 MHZ) with repeated measures on treatment was used. Change in cortisol levels was computed by taking the difference between the dialysate cortisol levels collected before and following treatment. Depth to muscle (MD), probe depth (PD), and pre-treatment dialysate cortisol concentrations (PreC) for both the treatment (Tx) and control (C) were used as covariates in the model. For all differences, the

level of significance was set at $P < .05$. Data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 17).

**Results**

Dialysate cortisol concentrations collected from interstitial fluid within the muscle were not affected by treatment or site. No significant difference in dialysate cortisol concentration change was found between treatment groups in the treated limb (Table 1; sham = 1.1 ± 7.5 ng/ml, 45 KHz = 1.1 ± 1.5 ng/ml, 1 MHz = 4.1 ± 7.8 ng/ml; $F_{2,22} = .34$, $P = .72$). No significant difference in dialysate cortisol concentrations during treatment was found when comparing the treated limbs and control limbs (treatment = 2.1 ± 6.2 ng/ml, control = 0.20 ± 5.9 ng/ml; $F_{1,22} = .9$, $P = .35$).

The results indicate that the depth of the muscle (MD) in the treatment group influenced dialysate cortisol concentration change (0.27 ± .06 cm; $F_{1,22} = 6.4$, $P = .02$). The results also indicate that the depth of the microdialysis probe (PD), inserted 1.23 ± .24 and 1.31 ± .23 cm in the control and treated limbs, respectively, influenced cortisol level change from the dialysate collected following treatment in the control group but not in the treatment group (control, $F_{1,22} = 4.1$, $P = .05$; treatment, $F_{1,22} = .78$, $P = .39$).

Furthermore, the dialysate cortisol levels prior to treatment influenced cortisol level change observed in the collected dialysate immediately following treatment in the control but not treated site (control = 15.8 ± 4.9 ng/ml, $F_{1,22} = 10.1$, $P = .004$; treated = 16.0 ± 3.5 ng/ml, $F_{1,22} = 1.1$, $P = .31$). The average dialysate cortisol concentrations following treatment in the control limbs and treatment limbs were 16.0 and 18.1 ng/ml, respectively.

**Discussion**

The lack of change in cortisol level following treatment with 45KHz and 1 MHz

ultrasound is in agreement with others.[19,30] Kuntz et al[30] reported similar results in a recent study comparing 1 MHz ultrasound with sham when attempting to deliver 10% hydrocortisone. Bare,[19] also attempting to deliver 10% hydrocortisone, reported no significant delivery following a similar treatment protocol.

Despite the outcomes of these studies, many authors are of the opinion phonophoresis is an effective tool for transdermal drug delivery.[15,18,21-24] Studies as early as 1954 by Fellinger and Schmid[36] indicated ultrasound could transport hydrocortisone for treatment of injury. Kleinkort and Wood[22] determined transdermal delivery of hydrocortisone was possible following his study in which ultrasound was used to treat various joints of the body. Griffin[9,10,16] furthered the discussion reporting successful delivery of hydrocortisone in studies using human subjects and swine tissue. More recently, Pribicevic[28] reported phonophoresis with 1% hydrocortisone cream was successful in treating shoulder injury when used in conjunction with massage and joint mobilization.

The literature appears to be inconclusive as to the effectiveness of transdermal drug delivery. However, a closer examination reveals the major difference between studies reporting drug delivery and those indicating no delivery occurred are the methods utilized by researchers to measure and determine the effectiveness of phonophoresis (Table 2).

Every author but one reporting drug delivery of hydrocortisone relied upon a clinical evaluation to determine success. Griffin[9,10,16,24] reported successful delivery of hydrocortisone through tissue analysis. However, delivery was determined utilizing chemical analysis of the swine tissue following treatment and a clinical evaluation was used following treatment in human subjects. Byl[21] too, reported drug delivery in swine tissue following ultrasound, but he delivered dexamethasone, a drug with a different molecular size. Kleinkort and Wood[22] relied

upon a clinical evaluation to determine if drug delivery had occurred. Patients reported feeling better or worse and functional tests were performed with advances credited to the treatment.

Researchers using an objective measure to determine drug delivery have not shown phonophoresis to effective. Bare [19] reported no treatment affect when they analyzed blood serum to evaluate increased levels of cortisol following treatment with 10% hydrocortisone cream. Utilizing the same treatment compound but examining cortisol levels in tissues from biopsies performed following treatment, Kuntz et al[30] also reported no treatment effect.

With the obvious correlation in methodology between studies reporting success and those indicating another result one could conclude phonophoresis does not facilitate actual transdermal drug delivery. Literature indicating delivery based upon methods other than chemical analysis could see patient improvement simply as a result of the application of ultrasound or time spent with the clinician. However, it is also possible the differences in reported outcomes are a result of the difficulty for researchers to accurately measure drug delivery.

Our ability to assert claims based upon detected cortisol levels was greatly affected by two factors: large variance between subjects and decreased power or small sample size in our analysis. Multiple subjects in each group demonstrated cortisol increases in either the control or the treatment limb during treatment. Multiple subjects in each group also demonstrated decreases in cortisol in collected dialysate in either the control or treatment limb. Studies in which results are varied as these can attempt to clarify results through increased testing of subjects. [30]

This variation could also be a result of the test used to evaluate drug delivery. For this study we utilized a cortisol enzyme immunoassay. The results of each assay were closely

analyzed. Standards contained in each sample kit to verify proper function of cortisol analysis were within anticipated range.

We also must consider our use of the microdialysis probe to collect dialysate. This is the first study of transdermal to utilize microdialysis. While testing of probes was done in vitro to determine expected outcomes, in vivo studies can affect outcomes by introducing differences among subjects. Consideration of subject variance, and attempting to control and decrease that variance, is essential.

The second purpose of this study was to evaluate how results are determined, developing a more sophisticated model through which we may more accurately assess drug delivery. This appears to be the first study to evaluate and control for all of the following parameters: pre-treatment cortisol levels in the target tissue, adipose thickness over the target tissue, and the exact depth from where cortisol measurements were taken; all of which proved to be significant factors in evaluating cortisol level change following treatment.

It is also necessary to decrease variance by controlling for these factors experimentally as well as statistically. With cortisol levels changing throughout the day it is necessary to begin testing with stable pre-level measurement. Normal plasma cortisol levels range from 50 to 230 ng/ml in the morning and can decrease to 30 to 150 ng/ml in the afternoon.[37] Several factors such as stress, diet, and fitness level can also strongly vary cortisol levels between subjects.[38] It is therefore recommended that testing be done on all subjects at approximately the same time of morning and minimizing the amount of activity and diet each subject will have experienced prior to reporting to the lab. The ability to analyze cortisol levels immediately prior to testing through a minimally invasive method also enables us to make more definitive conclusions about the

amount of drug actually being delivered through the skin. Microdialysis probes permit this testing.

Understanding sound waves and their reaction to certain mediums can further explain the need to monitor and control depth, not only from where we are taking measurements, but to the target tissue itself. As sound waves pass through tissues they decrease in energy, or attenuate, as a result of absorption of the sound wave.[39] Through Doppler imaging we were able to determine exact adipose tissue thickness and probe depth, providing a clearer analysis and explanation of expected sound wave activity. Subjects should be reasonably similar in adipose thickness over the treatment site to be used. If possible, collection depth should be similar in all subjects, minimizing variable attenuation of the soundwaves.

The frequency and intensity of ultrasound utilized during the experiment should also be monitored. Two main factors should be considered when determining ultrasound parameters: desired depth of soundwave penetration and tissue temperature. Lower ultrasound frequency is utilized for deeper tissues.[40] To ensure optimal soundwave depth, we utilized 1 MHz and 45 KHz. The intensity of the soundwave can directly affect the temperature of the treated tissue. Temperature increase in tissue can increase blood flow.[41] When utilizing ultrasound for TDD, increased blood flow in target tissues can decrease the ability of the desired medication to reach target tissues. As the medication passes the stratum corneum of the skin, increased blood flow can cause the drug to be taken into the blood stream more rapidly before reaching target tissues. This study utilized 1.0 $w/cm^2$ with the 1 MHz treatment and 0.56 $w/cm^2$ with the 45 KHz treatment, intensities specifically aimed to reduce increased blood flow for the treatment of acute injury.

**Conclusion**

In contrast to some previous studies yet comparable to others evaluating transdermal drug delivery, under the conditions of this study we detected no change in cortisol level due to treatment using 10% hydrocortisone cream. Our data does suggest that measuring cortisol levels prior to treatment, depth to the treated muscle, and the depth of the collection site can provide a clearer evaluation of efficacy of transdermal drug delivery.

Future studies involving phonophoresis should control for variance by using statistical control methods as this study does employing a General Linear Model with covariate analysis. In addition, future studies should control for variation experimentally in the following ways: First, researchers should understand the nature of ultrasound waves and their physiologic affect on various types of tissue, ensuring that the parameters including frequency, intensity, and time, are appropriate to achieve the desired affect. Second, one must not only monitor, but control the depth of the dialysis probe, understanding that increased adipose thickness as well as depth of collection does affect the ability of the researcher to accurately measure the delivered drug. And lastly, consider that factors such as time of day, stress, and fitness level can affect cortisol levels in the body.

Despite the inconsistencies in the literature, phonophoresis continues to be commonly used in treating musculoskeletal injury. Before a researcher or health care professional should accept or repudiate findings, it is important to understand possible explanations as to why discrepancy exists. Further studies are needed before definitive conclusions can be stated. However, understanding the sources of variation in measurement, testing, and evaluation can continue to improve, providing greater power and precision in the experimental results.

.

**References**


1. Kanikkannana N, Kandimalla K, Lamba SS, Singh M. Structure – activity relationship of chemical penetration enhancers in transdermal drug delivery. *Current Med Chem*. 1999;6:594-608.
2. Fedorczyk J. The role of physical agents in modulating pain. *Hand Ther*. 1997;10:110-121.
3. Panchagnula R. Transdermal delivery of drugs. *Indian* P*harmaco*. 1997;29:140-156.
4. Byl NN. The use of ultrasound as an enhancer or transcutaneous drug delivery: phonophoresis. *Phys Ther*. 1995;75:539-553.
5. Cameron MH. *Physical agents in rehabilitation: from research to practice*. Philadelphia: W.B. Saunders; 1999.
6. Cameron MH, Monroe LG. Relative transmission of ultrasound by media customarily used for phonophoresis. *Phys The*. 1992;72:142-148.
7. NG, K. Enhancing transdermal drug delivery with low-frequency ultrasound. *Drug Delivery Today*. 2004;9:913.
8. Fay, MF. Indications and applications for iontophoresis. *Today's OR Nurse*. 1989;11:10-16.
9. Griffin, JE. Low intensity phonophoresis of cortisol in swine. *Phys Ther*. 1968;48:1336-1344.
10. Griffin, JE. Ultrasonic movement of cortisol in pig tissues. *Am Phys Med.* 1963;42:77-85.
11. Johns LD. Nonthermal effects of therapeutic ultrasound: the frequency resonance hypothesis. *Athl Train.* 2002;37:293-299.

12. Gerscovich EO, Kurzrock EA. Acoustic streaming versus venous pseudoaneurysm in a scrotal mass. *Jour of Clin Ultrasound*. 2002;30:569-571.

13. Dyson M. Mechanisms involved in therapeutic ultrasound. *Physiotherapy*. 1987;73:116-120.

14. Tezel A, Sens A, Tuchscherer A, Mitragotri S. A theoretical analysis of low-frequency sonophoresis: dependence of transdermal transport pathways of frequency and energy density. *Pharm Res*. 2002;19:1841-1846.

15. Kleinkort JA, Wood AF. Phonophoresis with 1% vs 10% hydrocortisone. *Phys Ther*. 1980;60:307-308.

16. Griffin JE, Echternach JL, Price RE. Patients treated with ultrasonic driven hydrocortisone and with ultrasound alone. *Phys Ther* 1967;47:594-601.

17. Bertolucci LE. Introduction of anti inflammatory drugs by iontophoresis: double blind study. *Orthop Sports Phys Ther*. 1982;4:103-108.

18. Delacerda FG. A comparative study of three methods of treatment for shoulder girdle myofascial syndrome. *Jour Orthop Sports Phys Ther*. 1982;4:51-54.

19. Bare, AC. Phonophoretic delivery of 10% hydrocortisone through the epidermis of humans as determined by serum cortisol concentrations. *Phys Ther*. 1996;76:738-745.

20. Merino G. Frequency and thermal effects on the enhancements of transdermal transport by sonophoresis. *Control Release*. 2003;88:85-94.

21. Byl N, M$^{C}$Kenzie, Halliday B, Wong T, O'Connell J. The effects of phonophoresis with corticosteroids: a controlled pilot study. *J Orthop Sports Phy Ther.* 1993;18:590-599.

22. Kleinkort J, Wood F. Phonophoresis with 1% versus 10% hydrocortisone. *Phys Ther.* 1975;55:1320-1324.

23. Wing M. Phonophoresis with hydrocortisone in the treatment of temporomandibular joint dysfunction. *Phys Ther.* 1982;6232.

24. Griffin J, Echternach J, Price R, Touchstone J. Patients treated with ultrasonic driven hydrocortisone and with ultrasound alone. *Phys Ther.* 1980;47:594-601

25. Kahn J, Iontophorsis and ultrasound for post surgical for temporomandibular trismus and paresthesia. *Phys Ther.* 1978;60:307-308.

26. Kozanoglu E, Basaran S, Guzel R, Guler-Uysal F. Short term efficiency of ibuprofen phonophorsis versus continuous ultrasound therapy in knee osteoarthritis. *Swiss Med Wkly.* 2003;133:333-338.

27. Oziomek R, Perrin D, Harold D, Denegar C. Effect of phonophoresis of serum salicylate levels. *Med Sci Sports Exerc.* 1991;23:397-401.

28. Pribicevic M, Pollard H. A multi-modal treatment approach for the shoulder: a 4 patient case series. *Chiro Osteopath.* 2005;13:20.

29. Darrow H, Schulthies S, Draper D, Ricard M, Measom G. Serum dexamethasome levels after decadron phonophoresis. *Athl Train.* 1999;34:338-341.

30. Kuntz A, Griffiths C, Rankin J, Armstrong C, McLoughlin T. Cortisol concentrations in human skeletal muscle tissue after phonophoresis with 10% hydrocortisone gel. *Athl Train.* 2006;41:321-324.

31. Pitt W, Husseini G, Staples B. Ultrasonic drug delivery-a general review. *Expert Opin Drug Deliv.* 2004;1:37-56.

32. Smith N, Lee S, Maione E, Roy R, McElliott S, Shung K. Ultrasound medicate transdermal transport of insulin through in vitro human skin using novel transducer designs. *Ultrasound Med Biol.* 2003;8:1297-1304.

33. Mitrogotri S, Edwrds DA, Blankschtein D, langer R. A mechanistic study of ultrasonically-enhanced drug delivery. *J Pharm Sci*. 1995;84:697-706.

34. Mitrogotri S, Blankschtein D, langer R. Ultrasound-mediated transdermal protein delivery. *Science*. 1995;269:850-853.

35. Nyborg Wl. Physical mechanisms for biological effects of ultrasound. Washington, DC: Department of health, Education, and Welfare; 1978. FDA Publication 78-806.

36. Fellinger K, Schmid J. Khinik und therapie des chrnischen gelenkbuematismum. Vienna, Austria: Maudrich;1954:549-552.

37. Tietz, NW. Textbook of Clinical Chemistry. Saunders, 1968.

38. Brooks GA, Fahey TD, White TP, Baldwin KM. Exercise Physiology: Human Bioenergetics and Its Applications. Mayfield, 2000.

39. Laux D, Blasco H, Ferrandis JY, Hugon G, Despaux G, Leydier A, Mornet D. In vitro mouse model in duchenne muscular dystrophy diagnosis using 50-MHzz ultrasound waves. *Ultrasonics*. 2010;4:22.

40. Knight K, Draper D. Therapeutic modalities: the art and science. Philadelphia. Lippencott, Williams, Wilkons, 2008.

41. Draper D, Castel J, Castel C. Rate of temperature increase in human muscle during 1 MHz and 3 MHz continuous ultrasound. J Orthop Sports Phys Ther. 1995;22;142-150.

Table 1. Descriptive Statistics (N=31)

| | Sham | 45 KHz | 1 MHz |
|---|---|---|---|
| **Control** | | | |
| Pre Cortisol (ng/ml) | 14.2 ± 4.4 | 16.6 ± 3.1 | 16.5 ± 6.9 |
| Post Cortisol (ng/ml) | 15.8 ± 5.6 | 15.3 ± 2.9 | 16.9 ± 4.6 |
| Change* (ng/ml) | 1.6 ± 6.6 | -1.3 ± 2.2 | 0.4 ± 8.1 |
| Muscle Depth (cm) | 0.27± 0.07 | 0.27± 0.03 | 0.27± 0.04 |
| Probe Depth (cm) | 1.13± 0.19 | 1.27± 0.27 | 1.29± 0.24 |
| **Treated** | | | |
| Pre Cortisol (ng/ml) | 16.2 ± 4.6 | 16.1 ± 2.9 | 15.9 ± 3.1 |
| Post Cortisol (ng/ml) | 17.3 ± 6.7 | 17.2 ± 4.0 | 19.9 ± 7.6 |
| Change* (ng/ml) | 1.1 ± 7.5 | 1.1 ± 1.5 | 4.1 ± 7.8 |
| Muscle Depth (cm) | 0.28± 0.1 | 0.27± 0.03 | 0.27± 0.03 |
| Probe Depth (cm) | 1.38± 0.25 | 1.25± 0.27 | 1.33± 0.15 |
| **Range** | | | |
| **Control** | | | |
| Pre Cortisol (ng/ml) | 23.7 – 10.4 | 20.2 – 12.4 | 34.2 – 10.1 |
| | 29.9 – 11.0 | 20.3 – 12.0 | 26.0 – 11.6 |
| **Treated** | | | |
| Pre Cortisol (ng/ml) | 28.2 – 11.7 | 20.6 – 11.8 | 21.5 – 12.4 |
| Post Cortisol (ng/ml) | 34.7 – 10.4 | 24.4 – 11.8 | 35.4 – 11.6 |

*Change is the difference between pre-treatment cortisol and post treatment cortisol measurement

± represents standard deviation

Table 2. Previous In Vivo Phonophoresis Studies

| **Author** | **Medicine Delivered** | **Treatment Parameters** | **Area Treated** | **Measure Used to Verify Delivery** | **Affective Y / N** |
|---|---|---|---|---|---|
| Oziomek[27] 1991 | Myoflex | 1 MHz 1.5 w/cm$^2$ 5 minutes | Right Anterior Forearm | Serum Salicylate Concentration / Blood Draw | N |
| Bare[19] 1996 | 10% Hydrocortisone | 1 MHz 1.0 w/cm$^2$ 5 minutes | Forearm | Serum Cortisol Concentration / Blood Draw | N |
| Darrow[29] 1999 | Dexamethasone | 1 MHz 1.0 w/cm$^2$ 5 minutes | Left Forearm | Blood Draw | N |
| Kozanoglu[26] 2003 | 5% Ibuprofen Cream | 1 MHz 1.0 w/cm$^2$ 5 minutes | Knee Joint | Clinical Evaluation | N |
| Kuntz[30] 2006 | 10% Hydrocortisone | 1 MHz 1.0 w/cm$^2$ 7 minutes | Vastus Lateralis | Tissue Biopsy | N |
| Kleinkort[22] 1975 | 10% Hydrocortisone | 1 MHz 1-1.5 w/cm$^2$ 6 minutes | Various Structures | Clinical Evaluation | Y |
| Griffin[24] 1980 | 10% Hydrocortisone | 1 MHz 1.5 w/cm$^2$ 5 minutes | Various Structures | Clinical Evaluation | Y |
| Wing[23] 1982 | 10% Hydrocortisone | 1 MHz 1.0 w/cm$^2$ 8 minutes | TMJ Joint | Clinical Evaluation | Y |
| Byl[21] 1993 | Dexamethasone | 1 MHz 1.5 w/cm$^2$ 5 minutes | In Vivo Swine Tissue | Tissue Biopsy | Y |
| Pribicevic[28] 2005 | 1% Cortisone | Not specified | Shoulder | Clinical Evaluation | Undetermined |

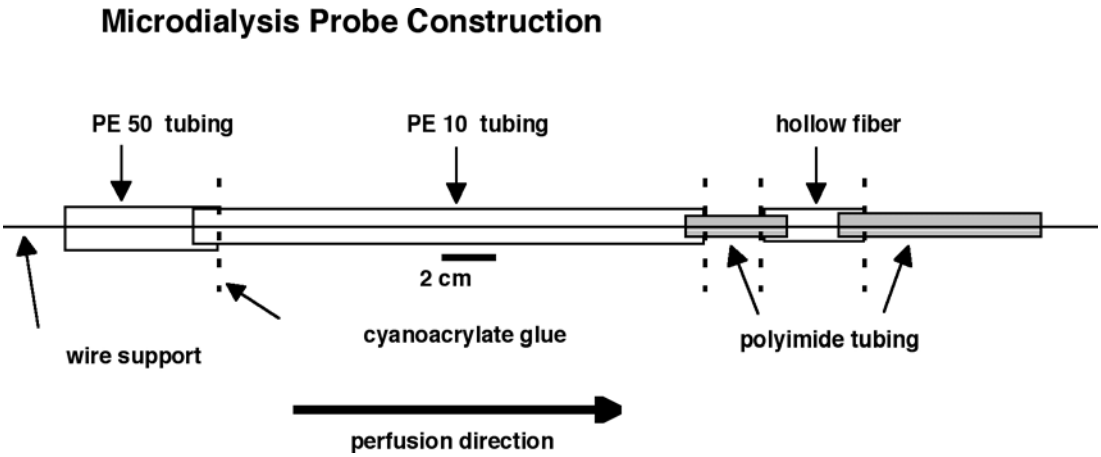



Figure 1. Schematic of Microdialysis Probe Used for Collection

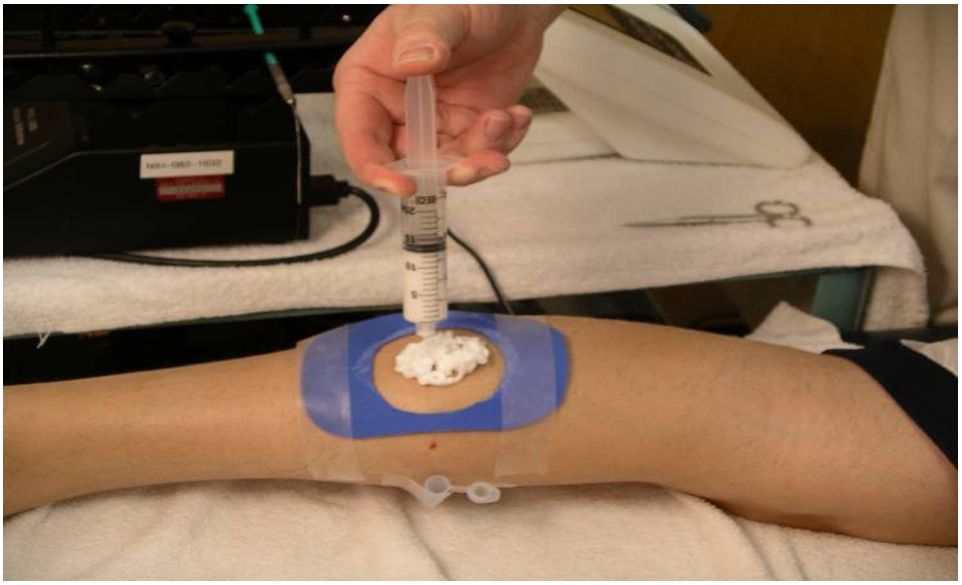


Figure 2.  Medicine Application Over Treatment Site

# Appendix A

## Prospectus

# Chapter 1

## Introduction

The use of medication in treatment protocols of musculoskeletal injury is a very common practice. However, with the use of various medications comes potential side affects. Long term use can result in organ damage as drugs are ingested, such as to the liver, kidneys, and stomach. Infections and excessive patient discomfort can occur during injection, as well prevalence of scar tissue build up in repeat injection sites. Questions arise as to the appropriate dosage amount to ensure adequate treatment following first pass through the digestive system, not to mention uncertainty in the amount of actual drug delivery reaching the target tissues. Alternative methods of drug delivery which can provide the patient with more effective and efficient means of medication are commonly explored. Transdermal delivery is one such method. Transdermal drug delivery is utilized to ease the complications that can occur with other drug delivery mechanisms.[1] Iontophoresis, medicated skin patches, and topical ointments are examples of frequently utilized transdermal drug delivery mediums. While the use of these tools is common, questions continue to arise regarding their efficacy. Researchers disagree in the amount of drug delivery to target tissues, patient response, and overall effectiveness of these methods.[1-23]

Phonophoresis, the use of ultrasound waves to deliver medication through the skin, is another frequently used method of transdermal medicine delivery.[4] However, it too, despite frequent application, contains many questions regarding its parameters of use including treatment duration, wave frequency, and overall effectiveness in delivering medication.

The use of sound waves propagated by ultrasonic devices as a therapeutic modality for the treatment of musculoskeletal trauma has been practiced for more than fifty years.[23] The variety of conditions for which ultrasound treatment is clinically used includes ligament, sprains,

muscular injury, inflamed tendons and tendon sheaths, lacerations, excessive scar tissue, inflamed and damaged joint capsules, bone regeneration and healing, delayed onset muscle soreness, and most recently thrombolysis.[24] The therapeutic benefits of ultrasound are classed into two categories: thermal and non-thermal. While the thermal effects and benefits of warming tissues are well documented, equally beneficial yet often overlooked and frequently misunderstood are the non-thermal or mechanical effects received during an ultrasound treatment.[23-26] Two common mechanical effects of ultrasound are cavitation, the expansion and contraction of cells when reacted upon by propagated sound waves, and acoustic streaming, the displacement of particles within and adjacent to the cell as a result of the ultrasound beam flow.[24]

It is via the mechanical effects of ultrasound that the drug delivery is reported to occur. These effects can alter cell wall permeability and cell function.[24] The rate of expansion and contraction of the cell, or cavitation, varies depending upon the frequency of the sound waves. Traditional ultrasound devices operate at frequencies of 1 MHz and 3 MHz. Phonophoresis for drug delivery typically utilizes the 1 MHz frequency treatment. However, as cellular expansion and contraction is increased, as opposed to rapid oscillation, the permeability of the cell wall is increased. This altered permeability, in conjunction with continuing acoustic streaming of particles, could affect the drug delivery rate during phonophoresis treatments.

Effective examination of drug amounts within the tissue during and following treatments could not only indicate success in delivery of desired medicine, but further explain mechanical effects on cellular function when utilizing ultrasound as a therapeutic modality. It is therefore important to evaluate what affect varying ultrasound frequency has on cellular permeability and function in effective transdermal drug delivery and how that delivery could be attributed to mechanical affects of ultrasound therapy.

**Research Questions**

1. Does altering ultrasound frequency affect transdermal drug delivery into muscle tissue?
2. Can this increased drug delivery be explained by non-thermal or mechanical effects of ultrasound in alteration of cell permeability and cellular function?

**Research Hypotheses**

The following hypotheses will guide this study.

1. Varying ultrasound frequency will alter transdermal drug delivery rate and depth of medicine into muscle.
2. Decreasing ultrasound frequency will increase drug delivery rate of medicine across the skin and into muscle.
3. Increased transdermal medicine delivery will occur at decreased ultrasound frequencies via mechanical properties of ultrasound therapy, including cellular cavitation and acoustic streaming.

**Operational Definitions**

Absorption – To take in, suck up, or imbibe.[70]

Acoustic Streaming – Physical forces of the sound waves that provide a driving force capable of displacing ions and small particles.[24]

Active Delivery Systems – Delivery systems in that require a physical force to facilitate the movement of drug molecules across the skin.[32]

Cavitation – The physical forces of the sound waves on microvenvironmental gases with fluid.[24]

Capillary – Any of the minute blood vessels, averaging 0.008 mm in diameter that connects the ends of the smallest arteries with the beginnings of the smallest veins.[70]

Cell Permeability – Cellular ability to allow the passage of fluids or substances across the cell membrane or wall.

Corticosteroids – Any of several steroid hormones secreted by the cortex of the adrenal gland or manufactured synthetically for the use as a drug. Drugs in this class are widely used to treat inflammatory illness such as arthritis, asthma, and dermatitis.[70]

Hypodermis – Deepest layer of skin tissue composed of white, fibrous connective tissue in which fat and elastic fibers are intermingled.[34]

Intracellular – Spaces within a single cell.

Ions – An atom or group of atoms that has lost one or more electrons and has a positive charge, or has gained one or more electrons and has a negative charge.[70]

Iontophoresis – An active transdermal drug delivery system that delivers ions and charged molecules across the skin into systemic circulation at an increase rate in a controllable manner via the use of electric current.[17]

Lag Time – The period of time between the application of a stimulus and the resulting reaction.

MHz (Megahertz) – One million cycles per second or $10^6$ hertz.[70]

Micro Streaming – The flow of interstitial fluids, or the pulsating of tissue particles associated with the a application of therapeutic ultrasound.[70]

Modality – The method of application or the employment of any therapeutic agent; limited usually to physical agent and devices.

Passive Delivery Systems – A mechanism by which a drug diffuses through the skin into the bloodstream using a simple concentration gradient as a driving force.[32]

Penetration – The act of breaking or crossing a barrier.

Percutaneous – Effected though the skin; describes the application of a medicated ointment by friction, or the removal or injection of a fluid by needle.[70]

Permeation – Penetration of and spreading throughout an organ, tissue, or space.

Phagocytosis – A three stage process by which phagocytes (neutrophils, monocytes, and macrophages) engulf and destroy microorganisms, other foreign antigens, and cell debris.[70]

Phonophoresis – The therapeutic application of ultrasound with a topical drug in order to facilitate transdermal drug delivery.[2]

Piezoelectric Effect – Application of an alternating current applied to a crystal resulting in changes of shape in tune with the electric field.[45]

Plasma - The liquid part of the lymph and blood consisting of serum, protein, and chemical substances. It acts as a transport for these substances as well as position waste products to various sites of clearance.

Sonophoresis – The use of ultrasonic energy created by low frequency sound waves to drive cutaneous transport of drug molecules.[33]

Stratum Corneum – The outer most layer of skin comprised on 10 to 20 cell layers. It is a fairly dense tissue composed of 20% water, lipids, and other proteins.[34]

Transdermal Drug Delivery – A delivery system technology that enables the passage of drug molecules across the intact skin utilizing passive and active methods.[32]

Transcellular – Through, across, over, or beyond the cell.[70]

Unstable Cavitation – The expansion and contraction of micro-bubbles within the cell at a rate that results in eventual explosion of the bubbles resulting in cellular damage.[24]

Ultrasound – A therapeutic modality that utilizing ultrasonic energy to emit thermal and mechanical responses within tissues.

Viable Dermis – The layer of skin located between the stratum corneum and the dermis. Water content of this tissue is 90%.[34]

Viable Epidermis – The layer of skin located directly beneath the dermis and subcutaneous tissue. It consists of a matrix of loose connective tissue comprised of fibrous protein and an amorphous ground substance.[34]

Wave Frequency – A disturbance, usually orderly and predictable, observed as a moving ridge with a definable frequency and amplitude. The number of repetitions of a produced wave.

## Assumptions

The following assumptions will guide this study:

1. Tissues among subjects will contain little variation in substance and resistance to applied treatments.
2. Tissues among subjects will respond with little variation in response to the treatment protocol including invasive microdialysis probe insertion.
3. Ultrasound devices utilized in the study will perform at desired and displayed frequencies.
4. Subjects will be honest in reporting any medical conditions that could affect results prior to participation.

## Delimitations

The study will be delimitated to:

1. Uninjured males and females between the ages of 18 and 30 from Brigham Young University and surroundings counties.
2. Individuals with no known drug allergies.
3. Individuals with no history of adverse reactions to applied heat therapy.
4. Individuals with no experience of decreased extremity sensation.
5. Individuals with no current illness or musculoskeletal injury.

# Chapter 2

## Review of Literature

The literature review for this study is organized into the following sections:

1. Search strategy
2. Background and significance
3. Transdermal drug delivery
    a. Benefits of transdermal drug delivery
    b. Methods of transdermal drug delivery
4. Delivery across the skin barrier
    a. Anatomy of the skin
    b. Passive delivery systems
    c. Active (mechanical) delivery systems
5. Ultrasound
    a. Benefits of ultrasound: thermal effects
    b. Benefits of ultrasound: non-thermal effects
6. Phonophoresis
    a. Mechanisms of phonophoresis
7. Sonophoresis
    a. Mechanisms of sonophoresis
8. Summery

### Search Strategy

PUBMed, SportsDISCUS, and CIHNAL databases were searched from using terms listed in Table 3. Additional literature was gathered from citations in articles, course texts and symposiums.

## Background and Significance

The utilization of drug therapy in the treatment of musculoskeletal trauma is a very common practice. These traumas are often treated with a variety of medications designed to reduce inflammation, relieve pain, relax muscle, and a number of other desired effects. While relief from musculoskeletal trauma can be obtained with medication, effective drug therapy is not without its difficulties. Delivery of these drugs is administered in three ways:

1. Oral ingestion, which can result in unpredictable levels of available drug in the blood stream following gastrointestinal metabolism, as well as other notable side effects such as altered liver, stomach, and kidney function following "first pass" digestion.[28-30]

2. Injection either administered intravenously or directly to the injury site, which can be painful leading to decreased patient compliance, as well, exposure to infection. Also, repeat injection sites often experience a prevalence of scar tissue, thus compromising tissue integrity and possible joint and muscle function.[30]

3. Topical application of medication, or transdermal delivery of drugs, which recently has seen a rise in both clinical and research trials largely due to difficulties with the first two mentioned modes of drug delivery.

## Transdermal Drug Delivery (TDD)

### *Benefits of Transdermal Delivery*

As recently as three years ago, 40% of all drugs in clinical trial were utilized via some form of transdermal delivery.[31] These drugs are commonly administered by topical cream, ointment, or skin patch. Transdermal drug delivery offers major benefits over other forms of delivery by:[1,3,6,28,31,32,33,34,35,36,]

1. Avoidance of first pass affects, producing steady state plasma levels improving bioavailability
2. Decreasing the dose administered as a result of liver bypass
3. Decreasing unwanted side affects
4. Decreasing gastrointestinal side affects
5. Ease of discontinuance in the case of adverse reactions
6. Increased patient compliance

Perhaps another reason accounting for the shift in research directed towards transdermal drug delivery is financial. The development of a new drug can near $250 million and take up to 15 years to reach the market. However, exploration of varying drug delivery of existing approved medications can be developed in half the time with 20% of the cost of new drug development.[1]

*Methods of Transdermal Drug Delivery*

Transdermal drug delivery is a system technology that enables the passage of drug molecules across intact skin.[32, 34] Skin is the most extensively and readily accessible organ in the body. It covers an area approximately 1.75 $m^2$ and receives one third of circulating blood in the body at any given time.[1] The potential for utilization of skin for pharmaceutical purposes has been recognized for many years.[37] Previously the skin was thought to be impenetrable. However, viewpoints have changed as further understanding regarding the complexity of this organ continues to grow.

Transdermal drug delivery occurs by one of two systems. First, via passive transdermal systems, also referred to as percutaneous absorption and second, the utilization of active transdermal systems or a mechanical force aiding in the delivery of drugs into tissues.[1,6,32]

Passive transdermal systems allow the drug to diffuse through the skin into the bloodstream using a concentration gradient to enhance or "drive" the medication into the tissue. This absorption can be defined as a penetration of a substance into various layers of skin and into systemic circulation.[1,32] This process occurs in three parts:

***Penetration*** - the entry of a substance into a particular layer

***Permeation*** - the penetration from one layer to another layer that is both functionally and structurally different from the first layer

***Absorption*** - the uptake of the substance into systemic circulation.

While the process of transdermal drug delivery appears to be very functional and easily available as an alternative in drug therapy, there are complications. The primary obstacle blocking the facilitation of this type of drug delivery is the very medium through which the process occurs: the skin.

**Delivery across the Skin Barrier**

*Skin Anatomy*

The skin can be considered to have four distinct layers of tissue: [34]

1. Non viable epidermis (Stratum Corneum)
2. Viable epidermis
3. Viable dermis (corium)
4. Subcutaneous connective tissue (hypodermis)

The stratum corneum or outer most layer of skin is the physical barrier to most substances that come in contact with the skin.[34] It is 10 to 20 cell layers, ranging from 1mm to 4mm in thickness and comprised of only 20 % water.[34, 38] Most other soft tissue within the body

is 70% water. It has been defined as a wall like structure comprised of protein bricks and a lipid mortar.[39] The stratum corneum is an effective and selective barrier to chemical permeation and the primary adversary in transdermal drug delivery.[31,40] Once through the stratum corneum, delivered medications will diffuse more effectively between the epithelial cells, hair follicles, and sweat glands.[41]

The second layer, viable dermis, is located between the stratum corneum and the dermis. It is thicker than the stratum corneum but is comprised largely of water, and thus is far more permeable.[34] The dermis or corium is just beneath the viable dermis. It consists of a matrix of loose connective tissue and fibrous protein.[34] The hypodermis or subcutaneous connective tissue technically is not considered a true part of the skin. It is comprised of white, fibrous connective tissue intermingled with blood and lymph vessels. The base of hair follicles, sweat glands, and cutaneous nerves are located in this region.[34]

*Passive Delivery Systems*

Passive transdermal permeation or "delivery" into the tissues via the skin occurs typically in one of three ways:[31,34]

1. Transcellular through the stratum corneum
2. Intercellular permeation through the stratum corneum
3. Transappendageal permeation via the hair follicles, sebaceous and sweat glands

Utilization of passive transdermal drug delivery can be managed successfully in drug therapy. The most common form of this type of delivery is seen with medicated skin patches. While this method is used, there are concerns as to the efficacy of passive delivery. Relatively few drugs passively penetrate the layers of the skin effectively enough to produce high

therapeutic levels in plasma. Difficulty also arises in delayed lag times for establishing a stable concentration. Often it takes several hours or longer to achieve an appropriate treatment state.[42] The application of this type of medication delivery can be limited and largely depends upon the purposes for which the drug is being used. Typically this type of delivery is seen with chronic dysfunction or ailments requiring long term medication. This prolonged treatment duration permits increased treatment times and initial decreases of a stable concentration of circulating medication. While it is possible to achieve a proper medicated state using passive transdermal systems with increased times, another concern arises. Because of high levels of capillarity located within the superficial surfaces of the skin, targeting exact tissue can be difficult with passive delivery.

As medications are passively absorbed into the skin, the delay enables the bloodstream to transport the slowly penetrating drug particles. If the purpose of medication is systemic, this type of delivery can be indicated. However, effectively targeting specific tissues with transdermal delivery can require a faster mode of delivery across the skin. In order to enhance skin permeability it is possible to utilize the second method of transdermal drug delivery: an active or mechanical force.

*Active (Mechanical) Delivery Systems*

Active or mechanical delivery systems require a physical force in which facilitation of movement of drug molecules across the skin occurs. By using some applied force such as an electric current or sound wave, active systems are able to deliver proteins and other large molecules such as medications to treat musculoskeletal trauma.

The two most commonly used forms of mechanical mechanisms in transdermal drug delivery are iontophoresis, the utilization of positively and negatively ions to deliver medication

across the skin barrier, and phonophoresis, the utilization of ultrasound waves to alter skin permeability allowing for increased transdermal penetration and permeation.

**Ultrasound**

*Indications of Use*

Physical agents are commonly used modalities in the treatment of musculoskeletal injury.[43] The use of ultrasound as a therapeutic modality for the treatment of musculoskeletal trauma has been practiced for more than fifty years.[23] The variety of conditions for which ultrasound treatment is clinically indicated includes ligament sprains, muscular injury, inflamed tendons and tendon sheaths, lacerations, excessive scar tissue, inflamed and damaged joint capsules, bone regeneration and healing, delayed onset muscle soreness, and most recently thrombolysis.[24,44] Over the past four decades researchers of ultrasound have tried to better explain what effect the use of this modality has upon structures within the body. While many questions have been answered, new questions have arisen. More recently, attention has been focused not only in tissues as a whole, but at the cellular level as well.

*Benefits of Ultrasound: Thermal effects*

The therapeutic benefits of ultrasound are classed in two categories: thermal and non-thermal. Ultrasound is capable of producing significant temperature changes in tissue.[43] These changes occur as the sound waves, produced by running an electric current through a synthetic crystal of lead zarconate, cause changes of shape within the crystal. This is known as the Piezoelectric effect.[24,45] These thickness changes are transmitted to the tissue as ultrasonic pressure waves emitting energy into the tissue.[44]

Experiments have reported varying levels of tissue temperature increase dependant upon treatment time and intensity. Research done by Draper has shown as much as .6$^{o}$ Celsius per

minute per watt per centimeter squared when using a 3 MHz frequency.[23,25,26] These thermal effects are often coupled with stretching, massage, and range of motion exercises all designed to help restore normal motion and activity.

The thermal benefits alone can promote and facilitate the healing of soft tissue, but also have been believed to be a cause of increasing transdermal drug delivery during phonophoresis. The influx of temperature caused by therapeutic ultrasound causes an increase in blood flow as the body aims to remain at its homeostatic state. This increased blood flow to damaged tissues transports blood cells and phagocytes used to aid in the restoration process of healing. As blood flow is drawn to the treatment area, the increased capillary activity enhances medicine transport by delivering the ultrasonically driven drug into the target tissues.

For many, it is this occurrence alone for which the basis of phonophoresis is formed. However, examination and understanding of the mechanical or non-thermal effects of therapeutic ultrasound can further explain other increased physiologic activity that occurs during treatment.

*Benefits of Ultrasound: Non-thermal Effects*

Not all of the therapeutic benefits of ultrasound are related to temperature increase. While the thermal effects and benefits of warming tissues are well documented, equally beneficial yet often overlooked are the non-thermal or mechanical effects received during an ultrasound treatment.[23-26] When reviewing the effectiveness of ultrasound, particularly with its role in transdermal drug delivery, it is important to examine both the thermal and non-thermal benefits. The non-thermal or mechanical effects of ultrasound at the cellular level can be equally beneficial in tissue healing and drug delivery. Several experiments have been successful in isolating the non-thermal from the thermal effects of ultrasound within cellular systems.[43,44,46] The most common and discussed mechanical effects are cavitation and acoustic streaming.[24,46,47]

The term cavitation was first introduced by Sir John Thornycroft in the early 20th century.[47] It is defined as the formation and life of bubbles in liquids.[47] It may also be referred to as "physical forces of sound waves on micro-environmental gases within fluid."[24] During therapeutic ultrasound treatment, sound waves travel through the coupling medium causing the expansion and contraction of the cells.[24] This event of compression and rarefaction causes microscopic gas bubbles in the tissue fluid to expand and contract. It is believed that continuing changes in pressure inside and around the cell can be detrimental to normal cellular function. It is possible for cellular destruction to occur if micro-bubbles continue to expand and contract causing "implosion", referred to as unstable cavitation.[24] While this phenomenon is not thought to occur at therapeutic levels of ultrasound, it is believed the pulsation of gas bubbles within the cell can not only affect cell wall permeability, but alter the function of the cell altogether. Some hypothesize the alteration of the cell wall can render medications more effective and allow for greater accumulation of medication to a more concentrated area.

Similar to cavitation, acoustic streaming affects the particles in and around the cell. When an ultrasound wave is transmitted through a fluid, the momentum transfer generates a steady motion of the fluid in the direction of the ultrasound beam flow .[46] This force created by the sound wave is capable of displacing ions and other small molecules.[48] Acoustic streaming can be divided into two areas: bulk streaming and micro-streaming. Bulk streaming occurs when an ultrasound beam propagates in a liquid and there is movement of the fluid in a single direction.[49] This event is less powerful than micro-streaming. Micro-streaming forms as "eddies" adjacent to the source of movement.[50]

Studies viewing the effects of acoustic streaming and cavitation on cells showed growth retardation of cells in vitro, increased protein synthesis[51,52], and membrane alterations.[53,54] This

would suggest that ultrasound can first injure the cell, retarding growth, and then initiates a healing response with increase protein production.[24] The original thought that ultrasound was a heating modality and thus only to be used in the later stages of tissue healing and regeneration is no longer the case. Performing therapeutic ultrasound in the acute stages of injury renders it possible to assist in the early stages of regeneration and repair. The application of ultrasound to areas marred by swelling can increase protein production and assists in tissues "stuck" within the inflammatory process.

It has previously been stated that ultrasound has two therapeutic effects, thermal and non-thermal. It has also been postulated that both of these affects can assist in healing and tissue repair. Now that both have been reviewed, it is important to discuss the interaction between the two. Is it possible to obtain the mechanical effects of ultrasound without exposing tissue to the thermal or heating affects? Research has indicated that sound waves traveling through a medium can cause an expansion and contraction of the cell. These effects can alter cell wall permeability and cell function.[24]

The extent to which this phenomenon occurs depends upon the frequency and intensity of the sound waves. Typically, therapeutic levels of ultrasound range from 1 to 3 MHz at frequencies around 1-2 $W/cm^2$. Depending upon desired heating levels treatment times may vary. By lowering the frequency of the sound wave, you will experience increased cavitation and potential micro-explosions of cells or unstable cavitation. As previously stated, typical therapeutic levels of ultrasound are performed at higher frequencies allowing for the benefits of acoustic streaming and cavitation without the detriments of excessive oscillation.

By lowering the intensity of the sound wave it is possible to obtain the mechanical benefits of ultrasound without increasing temperature.[24] Lower frequencies may also allow for

increased non-thermal effects without tissue heating. Previous beliefs that therapeutic ultrasound in the acute stage of injury is detrimental can be dispelled if proper treatment parameters are followed. Increased protein synthesis and cellular activity can be enhanced without tissues being exposed to vascular thermal reactions.

The practice of ultrasound remains a widely used modality for therapists in clinics, hospitals, and training rooms. The thermal effects promote increased healing of tissue and muscular relaxation, preparing the area for various treatments used to restore normal motion. Equally beneficial and more recently understood are the mechanical benefits of this modality. It is through these mechanical effects that the process of phonophoresis occurs.

**Phonophoresis**

*Mechanism of Phonophoresis*

Phonophoresis is the process by which ultrasound waves emitted during treatment push topically applied medication, mixed with conductive ultrasound gel, into the body.[2,45,55] This permeation and absorption across the stratum corneum and into the tissue is believed to occur as a result of the previously discussed mechanical effects of ultrasound. The utilization of ultrasound waves to enhance drug delivery is not a new concept. Studies reporting percutaneous absorption of medicine began in the 1950's by Fellinger and Schmid.[56] As a method of transdermal drug delivery, it too is believed to have advantages over traditional drug therapy. Phonophoresis is non-invasive and does not expose patients to painful injections or increased risk for infection. The application of the medication directly over the treatment site eliminates problems with oral ingestion such as gastric irritation and lessons the metabolic elimination by avoiding the liver.[2,17,57,58] Indications for phonophoresis use include injuries where traditional

medications would also be suggested such as muscle soreness, inflammation, bursitis, and tendonitis.[2,4]

*Effectiveness of Phonophoresis*

As with the general discussion regarding transdermal drug delivery, complication and doubts do arise as to the efficacy of phonophoresis. Numerous studies have been conducted assessing the clinical effectiveness of phonophoresis.[6-11] However, part of the difficulty in verifying the effectiveness of this technique stems from varying methods of evaluating the drug delivery. A majority of research conducted involving human subjects has relied heavily upon patient reported decreases in pain and dysfunction rather than direct tissue analysis.[18,19,59,60] Other in vivo studies reporting decreasing symptoms following treatment had uncontrolled variables such as varying patient treatments administered in conjunction with the phonophoresis treatments.[19] While many therapists do attest to its benefits, approximately 75% of reviewed research articles indicate some level of effectiveness, uncertainty with treatment parameters and delivery mediums continues to persist.[5,3,18,59,61,62]

As researchers continued to attempt to understand these parameters and how ultrasound waves can enhance transdermal delivery, focus was directed at the non-thermal or mechanical affects occurring during ultrasound treatments. Initially delivery and absorption was thought to be as result of increased thermal effects occurring during ultrasound. However, as studies began to examine this occurrence, the mechanical affects appeared to play the primary role in the drug delivery.[13,14] For example, in a study by Merino et al. ultrasound was delivered at 20 KHz at 15 $W/cm^2$ resulting in a $20^oC$ rise in temperature that resulted in a 35-fold increase in the level of delivered drug across porcine skin in vitro. However, when the same tissue was heated utilizing another form other than ultrasound to produce a thermal effect, the permeability of the drug

increased only 25 percent.[14] The decreased increase can be explained by the previously discussed methods of passive transdermal delivery such as absorption into sebaceous glands and hair follicles. But the increased levels of delivered medicine following ultrasound treatments is attributed to alteration within the stratum corneum as a result of delivered sound waves affecting cellular permeability.

While studies such as this and others performed have resulted in the general acceptance of phonophoresis as an effective active drug delivery method, questions continue to be asked. If the mechanical forces of sound waves on tissues within the stratum corneum and other dermal layers can be affect cellular permeability, can altering ultrasound treatment parameters result in increased transdermal drug delivery effectiveness?

**Sonophoresis**

*Mechanism of Sonophoresis*

Typical phonophoresis treatments occur within the range of 1 to 3 MHz. The growing understanding and acceptance of mechanical forces produced by ultrasound on cells such as cavitation and acoustic streaming help explain the mechanism of phonophoresis. However, this knowledge has led to the idea of further altering parameters with this type of active delivery to enhance transdermal delivery. By lowering the frequency of the sound waves it is hypothesized that the mechanical effects of the sound waves on cells will be enhanced. Low frequency ultrasound (20 - 500 KHz), or sonophoresis, is shown to be significantly more potent in enhancing skin permeability compared to the traditionally used frequencies of 1 and 3 MHz.[65] This is a direct result of the increased acoustic cavitation occurring in lower ultrasound frequencies. The number and size of cavitation bubbles appears to be inversely correlated with the ultrasound application frequency.[66] Mitragotri et al. demonstrated increased permeability of

skin tissue utilizing frequencies of 20 KHz with drugs used to enhance immune response.[28,67] Further studies by Mitragotri utilizing frequencies of 20 – 40 KHz have shown to increase the skin's permeability to insulin by 400 times.[68]

Low frequency ultrasound waves facilitate a considerable amount of cavitation as they pass through the tissue medium.[33] This ultrasonically produced formation of cavities promotes perturbation within the stratum corneum. The result is increased channels within the lipid structures, thus altering permeability of the stratum corneum to drug molecules.[69] This altered state results in increased penetration, permeation, and absorption of medication into the target tissues. Studies suggests that sonophoresis can enhance transport rates of molecules across the skin 100 fold.[63]

At this time the majority of research done to evaluate the utilization of sonophoresis as an active system in transdermal drug delivery has largely been devoted to insulin and other drugs used to enhance the immune system. The fact the many therapist currently utilize traditional frequencies of ultrasound or phonophoresis in the treatment of musculoskeletal trauma leads towards the need for continued exploration of sonophoresis as a more effective active transdermal drug delivery system.[3,35,36]

**Summery**

Transdermal drug delivery has become an important and often utilized means of drug administration.[29] The benefits of transdermal drug delivery over traditional methods of drug therapy are numerous:[1-6,12,31-33]

-avoidance of first pass metabolism

-ability to discontinue administration of drug by cessation of treatment

-control drug delivery for a longer time

-avoid gastrointestinal transit of drug component

-decrease need for injections

-less exposure to infection or drug reaction

-improved patient compliance

As the costs of drug production and lengthy process of drug approval continue to increase, new mediums for more effective delivery should continue to be examined. While the utilization of passive mediums for drug delivery such as topical ointments and skin patches continues to be prescribed, further research in areas of active delivery should be explored.

Traditional delivery methods of iontophoresis and phonophoresis continue to be recognized as effective mediums. However, as the mechanical properties of sound waves emitted via ultrasound devices continues to be understood, further evaluation is necessary. Not only to enhance understanding of non-thermal ultrasound effects, but also to increase utilization of those effects in more effectively aiding in the transmission, permeation, and absorption of drugs into target tissues. While current research in sonophoresis has largely been devoted towards drugs designed to facilitate general health, advancement into the treatment of musculoskeletal trauma can provide patients with safer and more effective drug therapy alternatives.

# Chapter 3

## Methods

### Design

A 2 X 2 X 3 factorial will be used. The first factor is the medicine that will be utilized during the study. The second factor is the intensity of ultrasound (1 MHz, 45 KHz, and sham). Subjects will receive one treatment from an assigned treatment group. Treatment protocol will consist of 10 minute sessions. Dependant variables will be recorded and consist of levels of medication collected during ultrasound delivery.

### Subjects

Thirty one healthy individuals will be recruited as subjects (10 subjects per treatment group). Prior to inclusion each subject will complete an information and health and history questionnaire. Subjects with recent or current history of general illness or history of decreased sensation to upper extremities will be excluded. Skin fold measurements at the probe insertion site located at the proximal extensor group of the elbow will be taken to evaluate adipose levels. Subjects with measurements greater than 10 mm thickness will be excluded. The institutional review board will approve this study and the subjects will give informed consent(Appendix A). Subjects will be assigned to a treatment group based upon random draw.

### Equipment

*Skin Fold Calipers*

*Ultrasound Devices and Coupling Gel.* 1 MHz ultrasound and sham ultrasound will be performed using a (Accelerated Care Plus, Reno, NV). 500 KHz and 20 KHz ultrasound will be performed using ultrasound transducers produced by the Brigham Young University Chemical Engineering Department(Provo, UT). Aquasonic 100 water soluble hypoallergenic ultrasound

transmission gel (Parker Laboratories, Inc, Fairfield, NJ) will be mixed with a topical cortisone gel.

*Intradermal microdialysis*

*Probe construction:* All components of the intradermal microdialysis probe are inert and non-reactive with human tissue. A 37 cm length of spring tempered stainless steel wire (0.002 inch diameter, Alan Baird Ind., NJ) is used to support the probe during construction and insertion into the tissue. The microdialysis probe consists of an 8 cm piece of polyimide tubing, 4 cm of hollow fiber dialysis tubing, 4 cm of polyimide tubing, 20 cm of PE 10 tubing, and 6 cm of PE 50 tubing. The polyimide tubing (0.0064 inch OD, Cole-Parmer, IL) is inserted 1 cm into the hollow fiber tubing and welded with cyanoacrylate glue on each end leaving 2 cm of exposed membrane. All welds are verified under a dissecting microscope. The hollow fiber microdialysis membrane is a regenerate cellulose membrane with an approximate 13,000 Dalton molecular weight cut-off (0D = 220 µm, Spectrum Laboratory, TX). After completion the probe is checked to see if it fits inside a 27 gauge needle. Probes are packaged individually and gas sterilized. The hollow fiber membrane allows equilibration of the perfusate solution with the interstitial fluid in the skin. Drug delivery analysis will be performed by Measurement of Cortisol (hydrocortisone) assay – Enzyme immunoassay (Immuno Biological Laboratories, Hamburg, Germany). 96 well microtiter plate. Measurement of Cortisol (hydrocortisone) assay – Enzyme immunoassay (Immuno Biological Laboratories, Hamburg, Germany). 96 well microtiter plate.

**Procedures**

Subjects will report to the Brigham Young University Human Performance Research Center for all screening and testing. Three skin fold measurements will be taken at the treatment site located at the proximal extensor group of the elbow with an average of the three measurements used

to determine subject inclusion. Qualified subjects will randomly draw to determine treatment group. Treatment groups consist of:

*Group One: 1 MHz 1.0 w/cm$^2$ – Treatment duration: 10 minutes*

*Group Two: Sham Ultrasound – Treatment duration: 10 minutes*

*Group Three: 45 KHZ – Treatment duration: 10 minutes*

*Probe placement:* Probes will be placed under aseptic conditions into the medial gastrocnemius muscle approximately 2 inches superior to the musculotendinous junction of the Achilles tendon with a 27-gauge needle used as a guide cannula.

A second probe will be inserted at a control site located on the approximate location on the opposite limb. Both probe entrance and exit sites on the skin will be separated by at least 6.0 centimeters. The guide cannula will be inserted horizontally in the dermis through the muscle. Probe depth and muscle penetration, as well as adipose and skin thickness will be verified and recorded with Doppler ultrasound imaging. After confirming that the needle has penetrated the muscle, the microdialysis probe will be fed through the guide cannula. Following insertion of the probe the needle will be removed with the probe left in place.

After placement, the probes will be perfused with 0.9% sterile saline at a rate of 10µl/min with a Harvard infusion pump for 70 minutes. The purpose of this period is to allow tissue to recover from needle and probe insertion. After insertion, the volume of dialysate (fluid coming out of the probe) will be monitored for five minutes. If this volume is significantly lower than expected (indicating a leak) the probe will be replaced. At minute 70 of the recovery period we will alter the infusion rate to 5µl/min and dialysate will be collected for 20 minutes to determine pre-treatment tissue cortisol levels. The perfusion rate will remain constant for the remainder of the treatment.

*Drug Delivery Treatment.* After 20 minutes of collecting dialysate at 5µl/min, the vials will be replaced. We will then place the prepared medicated ultrasound coupling gel on a treatment area 2 times the size of the ultrasound head.

We will use a pre fabricated template to isolate the treatment area and ensure consistent treatment size. The ultrasound operator will guide the sound head for the duration of the 10-minute treatment at a rate of approximately 4 cm per second. During the treatment, dialysate from the intramuscular microdialysis probe will be collected for analysis.

Following the treatment, subjects will remain in the prone position for an additional 10 minutes while dialysate continues to be collected at a rate of 5µl/min in an effort to ensure that medication delivered during the ultrasound treatment will pass to the vials. After 10 minutes the saline perfusion will be terminated and the collected fluid will be stored in the laboratory freezer. We will remove the ultrasound template and clean medicated ultrasound gel from the dermis. We will remove the intramuscular microdialysis probes without discomfort and portal sites within the tissue will be treated with triple antibiotic and covered with a band aid. We will give subjects a basic wound care guide with contact information should any questions arise.

**Statistical Analysis**

We will use a 2 X 2 X 3 (condition x time x treatment) ANCOVA to analyze change in cortisol levels in samples collected before and after treatment in both treatment sites and control sites. Bonferroni post hoc multiple comparison test will be used to examine individual differences in cortisol levels between the 3 treatments. For all differences, the level of significance will be set at $P < .05$. Data will be analyzed using MINITAB software.

**References**

1. Panchagnula R. Transdermal delivery of drugs. *Indian Jour of Pharmacology*. 1997;29:140-156.

2. O'Rourke K, Framel S. Phonophoresis. *Current Issues in Physical Therapy*. 2002.

3. Byl NN. The use of ultrasound as an enhancer or transcutaneous drug delivery: phonophoresis. *Physical Therapy*. 1995;75(6):539-553.

4. Cameron MH. *Physical agents in rehabilitation: from research to practice*. Philadelphia: W.B. Saunders; 1999.

5. Cameron MH, Monroe LG. Relative transmission of ultrasound by media customarily used for phonophoresis. *Physical Therapy*. 1992;72(2):142-148.

6. Fedorczyk J. The role of physical agents in modulating pain. *Jour of Hand Therapy*. 1997;10:110-121.

7. Kleinkort JA, Wood AF. Phonophoresis with 1% vs 10% hydrocortisone. *Physical Therapy*. 1980;60:307-308.

8. Griffin JE, Echternach JL, Price RE. Patients treated with ultrasonic-driven hydrocortisone and with ultrasound alone. *Physical Therapy* 1967;47:594-601.

9. Bertolucci LE. Introduction of anti inflammatory drugs by iontophoresis: double blind study. *Journ of Orthop Sports Phys Ther*. 1982;4:103-108.

10. Harris PR. Iontophoresis: clinical research in musculoskeletal inflammatory conditions. *Journ of Orthop Sports and Phys Ther*. 1982;4:109-112.

11. Delacerda FG. A comparative study of three methods of treatment for shoulder girdle myofascial syndrome. *Jour Orthop Sports Phys Ther*. 1982;4:51-54.

12. NG, K. Enhancing transdermal drug delivery with low-frequency ultrasound. *Drug Delivery Today*. 2004;9(21):913.

13. Tezel, A et al. A theoretical analysis of low-frequency sonophoresis: dependence of transdermal transport pathways of frequency and energy density. *Pharm Res*. 2002;19:1841-1846.

14. Merino, G. Frequency and thermal effects on the enhancements of transdermal transport by sonophoresis. *Jour of Control Release*. 2003;88:85-94.

15. Fay, MF. Indications and applications for iontophoresis. *Today's OR Nurse*. 1989;11:10-16.

16. Griffin, JE. Low intensity phonophoresis of cortisol in swine. *Physical Therapy*. 1968;48:1336-1344.

17. Griffin, JE. Ultrasonic movement of cortisol in pig tissues. *Am Jour Phys Med.* 1963;42:77-85.

18. Wing, M. Phonophoresis with hydrocortisone in the treatment of temporomandibular joint dysfunction. *Physical Therapy*. 1982;62:32-33.

19. Bare, AC. Phonophoretic delivery of 10% hydrocortisone through the epidermis of humans as determined by serum cortisol concentrations. *Physical Therapy*. 1996;76:738-745.

20. Median VM, Docker MF. Phonophoresis of hydrocortisone with enhancers: an acoustically designed model. *Inter Nat Jour of Pharm*. 1998;170:157-168.

21. Hasson SM. Dexamethasone iontophoresis: effect on delayed muscle soreness and muscle function. *Canadian Jour of Sport Sci*.1992;17:8-13.

22. Li LC. The efficiency of dexamethasone iontophoresis for the treatment of rheumatoid arthritic knees: a pilot study. *Arthritis Care and Research*. 1996;9:126-132.

23. Draper DO, Castel JC, Castel D. Rate of temperature increase in human muscle during 1 MHz and 3 MHz continuous ultrasound. *Jour of Sport and Phys Ther*. 1995;22(4):142-149.

24. Johns LD. Nonthermal effects of therapeutic ultrasound: the frequency resonance hypothesis. *Jour of Athl Train*. 2002;37(3):293-299.

25. Chan AK, Myrer JW, Measom GJ, Draper DO. Temperature changes in human patellar tendon in response to therapeutic ultrasound. *Jour of Athl Train*. 1998;33(2):130-135.

26. Draper DO, Schulthies S, Sorvisto P, Hautala AM. Temperature changes in deep muscles of humans during ice and ultrasound therapies: an in vivo study. *Jour of Sport and Phys Ther*. 1995;21(3):153-157.

27. Gilman AG et al. *Goodman and Gilmans The Pharmacological Basis of Therapeutics, 9th ed*. M^C Graw – Hill: New York, NY. 1996.

28. Wu J, Chappello J, Yang J, Weinmann L. Defect generated in human stratum corneum specimens by ultrasound. *Ultrasound in Med and Bio*. 1998;25(5):705-710.

29. Rosado C, Rodrigues LM. Solvent effects in permeation assessed in vivo by skinsurface biopsy. *BMC Dermatology*. 2003;3(5):1-6.

30. Tezel A, Sens A, Tuchscherer J, Mitragotri S. Frequency dependence of sonophoresis. *Pharm Research*. 2001;18(12):1694-1700.

31. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. *European Jour of Pharm Sci*. 2001;14:101-114.

32. *Transdermal drug delivery systems: a technical and strategic outlook.* Frontline Strategic Consulting, Inc. 2002.

33. Median VM, Michniak BB. Emerging technologies in transdermal therapeutics. *Amer Journ of Therapeutics*. 2004;11:312-316.

34. Kanikkannana N, Kandimalla K, Lamba SS, Singh M. Structure – activity relationship of chemical penetration enhancers in transdermal drug delivery. *Current Med Chem*. 1999;6:594-608.

35. Ciccone CD, Robinson AJ, Snyder-Mackler L. *Clinical Electrophysiology: Electrotherapy and Electrophysiologic Testing, 2ed*. Baltimore, Williams and Wilkens. 1995:331-358.

36. Castello CT, Jeske AH. Iontophoresis: applications in transdermal medication delivery. *Physical Therapy*. 1995;75:554-562.

37. Asbill CS, El-Kattan AF, Michniak BB. Enhancement of transdermal drug delivery: chemical and physical approaches. *Crit Rev Ther Drug Carrier Syst*. 2000;17:621-658.

38. Guyton AC, Hall JE. *Textbook of Medical Physiology, 10th ed*. W.B. Saunders: Philadelphia, PA. 2000.

39. Williams ML, Elias PM. The extracellular matrix of stratum corneum: role of lipids of normal and pathological function. *Crit Rev Ther Drug Carr Sys*. 1989;3:95-122.

40. Scheuplein RJ, Blank IH. Permeability of the skin. *Phys Rev*. 1971;51:702-747.

41. Bronaugh RI, Maibach HI. *In vitro percutaneous absorption. Fundamentals and Applications*. Ann Arbor, MI CRC Press, 1991:26-30.

42. Arndts D, Arndts K. Pharmacokinetics and pharmacodynamics of transdermally administered clonidine. *Eur Jour Clin Pharmacol*. 1984;26:79-85.

43. Kitchen SS, Partridge CJ. A review of therapeutic ultrasound. *Physiotherapy*. 1990;76:593-600.

44. Binder A. Is therapeutic ultrasound effective nitrating soft tissue lesions. *Br Med Jour*. 1985;290:512-514.

45. Saxena J, Scharma N, Makoid MC, Banakar UV. Ultrasonically medicated drug delivery. *Jour of Biomaterials Applications*. 1993;7:277-295.

46. Gerscovich EO, Kurzrock EA. Acoustic streaming versus venous pseudoaneurysm in a scrotal mass. *Jour of Clin Ultrasound*. 2002;30:569-571.

47. Dyson M. Mechanisms involved in therapeutic ultrasound. *Physiotherapy*. 1987;73:116-120.

48. Nyborg WL. *Physical mechanisms for biological effects of ultrasound*. Washington, DC: Department of Health, Education, and Welfare; 1978: FDA Publication 78-8062.

49. Wu J, Winkler AH, O'Neil TP. Effect of acoustic steaming on ultrasonic heating. *Ultrasound Med Bio*. 1994;20:195-201.

50. Williams AR. *Ultrasound: biological affects and potential hazards*. London, England: Academic Press;1983.

51. Ross P, Edmonds PD. Ultrasound induced protein synthesis as a result of membrane damage. *Jour Ultrasound Med*. 1983;2(suppl):47.

52. Webster DK, Pond JB, Dyson M, Harvery W. The role of cavitation in the in vitro stimulation of protein synthesis in human fibroblasts by ultrasound. *Ultrasound Med Biol*. 1978;4:343-351.

53. Anderson DW, Barrett JT. Depression of phagocytosis by ultrasound. *Ultrasound Med Biol*. 1981;7:267-273.

54. Pinamonti S, Gallenga PE, Masseo V. Effects of pulsed ultrasound on human erythrocytes in vitro. *Ultrasound Med Biol*. 1982;8:631-638.

55. American Physical Therapy Association. *Guide to Physical Therapist Practice, 2nd ed*. Alexandria, VA: APTA; 2001.

56. Fellinger K, Schmidt J. *KliniK und therape des chronischem*. 1954. Vienna, Austria: 549-554.

57. Starkey, C. *Therapeutic Modalities 2nd ed*. 1999. Philadelphia, PA: F.A.Davis.

58. Griffin JE, Touchstone JC, Liu ACY. Ultrasonic of cortisol into pig tissues, II:movement into paravertebral nerve. *Am Jour Phys Med*. 1965;44:20-25.

59. Kleinkort JA, Wood F. Phnophoresis with 1% versus 10% hydrocortisone. *Physical Therapy*. 1975;55:1320-1324.

60. Kahn J. Iontophoresis and ultrasound for post surgical temporomandibular trismus and paresthesia. *Physical Therapy*. 1980;60:306-308.

61. Goddard DH. et al. Ann Rheum Dis. 1983;42:582-584.

62. Pratzel H et al. J Rhematol. 1986;13(6):1122-1125.

63. Mitragotri S, Blankschtein D, Langer R. Ultrasound mediated transdermal protein delivery. *Science*. 1995;269:850-853.

64. Gaertner W. Frequency dependence of acoustic cavitation. *Jour Acoustic Soc Am*. 1954;26:977-980.

65. Mitragotri S et al. Determination of threshold energy dose for ultrasound-induced transdermal srug transport. *Jour of Control Release*. 2000;63(1-2):41-52.

66. Mitragotri S, Kost J, Langer R. Non invasive drug deliver and diagnostics using low frequency sonophoresis. *Recent Advances and Research Updates*. 2000;1:43-48.

67. Merino G, Kalia YN, Guy RH. Ultrasound-enhanced transdermal transport. *Jour Pharm Sci*. 2003;92:1125-1137.

68. Chien YW. *In novel drug delivery systems: revised and expanded edited* by: Chien WY. New York: Marcel Dekker. 1992:310-380.

69. Mitragotri S, Kost J. Low frequency sonophoresis a review. Advance *Drug Delivery Reviews*. 2004;56:589-601.

70. *Taber's Cyclopedic Medical Dictionary 19th Edition*. F.A. Davis Company: Philadelphia, PA. 2001.

# Appendix B

## Additional Methods

Table B 1. Table of Tables and Figures of Additional Methods

Table B 2. Research Questions and Statistical Analysis Procedures Performed

**1. Is ultrasonic frequency a significant parameter in enhancing the delivery of cortisone (using 10% hydrocortisone cream)?**

a. I used a General Linear Model with ANCOVA and repeated measures to determine if a group difference existed and to see what covariates used in analysis were significant:
   i. In SPSS
      1. Analysis Variance – Repeated Measures Model
      2. Covariates were: Muscle Depth Treatment (MDTx), Probe Depth Treatment (PDTx), Pre-Cortisol Treatment (PreCTx), Muscle Depth Control (MDC), Probe Depth Control (PDC), Pre-Cortisol Control (PreCC)
      3. Response Variable was: change
      4. Factor Variable was: sham, 45 KHz, 1 MHz

**2 X 3 Analysis of Variance Table**

| Source Term | DF | Sum of Squares | Mean Square | F-Ratio | Prob Level |
|---|---|---|---|---|---|
| Cortchange | 1 | 16.531 | 16.531 | .904 | .352 |
| Cortchange*MDTx | 1 | 117.807 | 117.807 | 6.443 | .019 |
| Cortchange*PDTx | 1 | 14.320 | 14.320 | .783 | .386 |
| Cortchange*PreCTx | 1 | 19.446 | 19.446 | 1.064 | .314 |
| Cortchange*MDC | 1 | 24.372 | 24.372 | 1.333 | .261 |
| Cortchange*PDC | 1 | 74.884 | 74.884 | 4.096 | .055 |
| Cortchange*PreCC | 1 | 184.413 | 184.413 | 10.086 | .004 |
| Cortchange*Group | 2 | 12.390 | 6.195 | .339 | .716 |
| Error(Cortisolchange) | 22 | 402.245 | 18.284 | | |

There was no difference in change between the control and treatment limb ($F_{1,22} = .9$, $P = .352$). There was also no difference in change between any of the three treatment groups ($F_{2,22} = .34$, $P = .716$). Since there was no indication of difference between treatments or groups, no further analysis or statistical tests were performed.

**2. Do pre-treatment cortisol levels, depth to the treated muscle, and depth of the microdialysis collection probes influence the analysis determining if drug delivery occurred?**

The following measured covariates were significant, appearing to influence the post cortisol levels in the collected dialysate: Muscle Depth ($F_{1,22} = 6.4$, $P = .019$), Probe Depth ($F_{1,22} = 4.1$, $P = .055$), and Pre Cortisol Level ($F_{1,22} = 10.1$, $P = .004$). Despite the fact that these covariates were significant on either the treatment or control, we should still continue to measure and control at both the treatment and control sites

Table B 3. Subject Information and Data Collection Form

Subject Information Form

Date:

Subject Number _____

Treatment Group_____

Number in Group_____

Ultrasound Imaging Data

Treatment Site

Depth to Muscle _____

Probe Depth _____

Control Site

Depth to Muscle _____

Probe Depth _____

Note any requested variations on treatment protocol:
(Excessive movement, discomfort, pain, etc)

Figure B 1. Institutional Review Board Application

# Application for the Use of Human Subjects Application Information

| | | | |
|---|---|---|---|
| 1. The Effects of Low Frequency Ultrasound in Transdermal Drug Delivery | | | |
| 2. Principal Investigator: Aaron Wells | | 3. Contact Person: (if different from PI): | |
| Title: ATC,L PhD Student | Dept: Exercise Science | Title: | Dept: |
| 1861 W 870 N Pleasant Grove, UT 84062 | | Address (+ Zip): | |
| Phone: (801) 796-6758 | Email: Wells@byu.edu | Phone: | Email: |
| 4. Co-Investigator(s): David Draper (Name & Affiliation) Professor – Brigham Young University | | | |
| 5. Research Originated By: (Check One) ☐ Faculty **X** Student ☐ Staff | | | |
| 6. Research Purpose: ☐ Grant **X** Dissertation ☐ Thesis ☐ ORCA Scholarship (Check All that Apply) ☐ Other ☐ Honors Thesis ☐ Course Project: Which Course? | | | |
| 7. Correspondence Request: ☐ Mail **X** Call for Pick-Up | | | |

## Research Study Synopsis

1. Short Study Description: Therapeutic drug use can have undesirable side affects, whether it is possible organ and tissue damage due to oral medication or pain, infection, and scarring with injection. Questions also arise as to the efficacy of drug delivery to target tissues as well as other systemic affects with prolonged drug use. The use of ultrasound in conjunction with transdermal drug delivery is common. However there are doubts about its success in delivering medication to the desired treatment areas. The purpose of this study is to see if altering the frequencies of therapeutic ultrasound increases transdermal drug delivery.

**Study Length**

What is the duration of the study? The study will take approximately 75 days to complete

**Location of Research**

a. Where will the research take place? Data collection will take place in the Health and Human Performance Modality Laboratory in 126 RB

b. Will the PI be conducting and/or supervising research activity at any sites not under the jurisdiction of the IRB? No

**4. Subject Information**:

a. Number of Subjects: 30 b. Gender of Subjects: Male and Female

c. Ages – Varies

**5. Potentially Vulnerable Populations:** (Check All that Apply)

☐ Children ☐ Pregnant Women ☐ Cognitively Impaired ☐ Prisoners ☐ Institutionalized **X** Faculty's Own Students ☐ Other. Please describe:

**6. Non-English Speaking Subjects**

a. Will subjects who do not understand English participate in the research: ☐ Yes **X** No

b. If yes, describe your resources to communicate with the subjects:

c. Into what language(s) will the consent form be translated:

**7. Additional Subject Concerns**

a. Are there cultural attitudes/beliefs that may affect subjects in this study? ☐ Yes **X** No

b. If yes, please describe attitudes and how they may affect subjects.

**8. Dissemination of Research Findings**

a. Will the research be published? **X** Yes ☐ No If yes, where if known?

b. Will the research be presented? **X** Yes ☐ No If yes, where if known?

**9. External Funding**

a. Are you seeking external funding? ☐ Yes **X** No What agency?

b. Have you received funding? **X** Yes ☐ No c. Dollar amount? $3000

**10. Method of Recruitment: (Check All that Apply)**

**X** Flyer **X** Classroom Announcement ☐ Letter to Subjects ☐ Third Party ☐ Random ☐ Other

**11. Payment to Subjects**

a. Will subjects be compensated for participation? **X** Yes ☐ No If yes, please indicate amount: $30

b. Form of Payment: **X** Cash ☐ Check ☐ Gift Certificate ☐ Voucher ☐ 1099 ☐ Other

c. Will Payment be prorated? ☐ Yes **X** No If yes, please explain:

d. When will the subject be paid? **X** Each Visit ☐ Study Completion ☐ Other

**12. Extra Credit**

a. Will subjects be offered extra credit? ☐ Yes **X** No

b. If yes, describe the alternative:

**13. Risks**: Identify all potential risks/discomforts to subjects. The insertion of the microdialysis probe may cause small discomfort. The application of therapeutic ultrasound may produce mild warmth.

**14. Benefits:**

a. Are there direct benefits to participants? ☐ Yes **X** No If yes, please list.

b. Are there potential benefits to society? **X** Yes ☐ No If yes, please list. The utilization of alternate routes of drug delivery can increase patient compliance, reduce drug costs, and increase safer delivery of medication to tissues.

**15. Study Procedures**:

a. What will be the duration of the subjects' participation? A one time two hour visit

b. Will the subjects be followed after their participation ends? ☐ Yes **X** No If yes, please describe.

c. Describe the number, duration and nature of visits/encounters. Students will arrive at the laboratory and fill out informed consent. Subjects will then have adipose tissue thickness measured to ensure qualification. They will then have the microdialysis probe inserted and undergo a 90 minute recovery time. Treatment application lasting 10 minutes will be performed. Subjects will then have probe removed and insertion site will be cleansed and

covered. Subjects will be given a wound care sheet with emergency contact information and paid for their participation. They will then be excused.

d. Is the study **X** Therapeutic? □ Non-therapeutic?
e. List all procedures that will be performed to generate data for the research.
Subjects will report to the Brigham Young University Human Performance Research Center for screening and testing. Skin fold measures will be taken at the treatment site located at the proximal wrist extensor group. The average of three measurements will be used to determine insertion site and depth needed to ensure penetration of the muscle. Subjects will randomly draw to determine treatment group. Treatment groups consist of:

Group One: Sham Ultrasound– Treatment duration: 10 minutes
Group Two: 45 KHz .056 w/cm$^2$ – Treatment duration: 10 minutes
Group Three: 1 MHz 1.0 w/cm$^2$ 50% Duty Cycle – Treatment duration: 10 minutes

***Probe Placement***. Under sterile conditions the probe is placed in the medial gastrocnemius muscle distal to the knee at a depth no less than 1 cm into the muscle with a 27-gauge needle used as a guide cannula. A second probe will be inserted at a control site located in the gastrocnemius muscle of the opposite leg in the approximate location of the treatment site. The entrance and exit sites on the skin are separated by at least 2.5 cm. The guide cannula is inserted horizontally in the dermis and the microdialysis probe is fed through the guide cannula. The needle is removed with the probe left in place. Measurements of tissue to estimate adipose and muscle depth are performed with skin fold calipers. Probe depth and muscle penetration will be verified utilizing ultrasound imaging following insertion. After placement, the probes are perfused with 0.9% saline at a rate of 10 µl/min with a Harvard infusion pump. A 90 minute recovery period after probe insertion and associated soft tissue trauma is required to allow local skin blood flow to return to baseline, as well as ensure normal Cortisol levels present in the muscle before data measurements can be made.

Placement of the microdialysis probes into the muscle may produce some transient pain or discomfort. Risks associated with insertion of the microdialysis probe include infection and irritation in the skin but these are reduced by observance of proper sterile techniques. The microdialysis probes will be sterilized using clinically employed techniques at the Human Performance Research Center (gas sterilization).

The microdialysis probe is placed in the muscle using a guide cannula (a 27-gauge needle under the skin). As such, the risk of damaging the probe and incurring a leak or break is minimized. After insertion we perfuse each probe with sterile saline at 5 µl/min and monitor the volume of dialysate (fluid coming out of the probe) for five minutes. If this volume is significantly lower than expected (indicating a leak) the probe is replaced.

***Drug Delivery Treatment***

Following probe insertion and 90 minute recovery period, the prepared medicated ultrasound coupling gel will be placed on a treatment area 2 times the size of the ultrasound head utilized for the specific treatment. A pre-fabricated template will be used to isolate the treatment area and ensure appropriate treatment area and size. The ultrasound operator will guide the sound head for the duration of the 10 minute treatment at a rate of approximately 4 cm per second, advising the subject to immediately report any unexpected discomfort during the session. During the treatment, perfusate from the intramuscular microdialysis probe will be collected into test tubes for analysis. At the conclusion of the treatment, subjects will remain in the prone

position for an additional 10 minutes to permit dialysate collection. Following that period, the subject will have the ultrasound template removed and medicated ultrasound gel cleansed from the dermis. Intramuscular microdialysis probes will be removed without discomfort and portal sites within the tissue will be treated with triple antibiotic and covered with a band aid. Subjects will then be given a basic wound care guide with contact information should any questions arise. Subjects will then be given a $35 honorarium for participation and excused.

16. Informed Consent:
    a. Are you requesting Waiver or Alteration of Informed Consent? ☐ Yes **X** No If yes, please fill out the waiver of informed consent and attach it.
    b. Briefly describe your process to obtain consent: An informed consent form describing testing purpose, procedures, and possible risks will be distributed to subjects upon arrival to the laboratory. Subjects will be given time to read the informed consent form and be asked if they have any questions. They will then be asked to sign the consent.
17. Confidentiality:
    a. Are the subject's social security number, BYU ID number or any identifier (other than study number and initials) being sent off site? ☐ Yes **X** No If yes, describe and explain reasons.

    b. Will any entity other than the investigative staff have access to medical, health or psychological information about the subject? ☐ Yes **X** No If yes, please indicate who.

    c. Briefly describe provisions made to maintain confidentiality of data, including who will have access to raw data, what will be done with the tapes, etc. All records and data will be kept by the principle investigator under lock in the office of the principle investigator. All records kept on computer of the principle investigator will be password protected.
    d. Will raw data be made available to anyone other than the PI and immediate study personnel? ☐ Yes **X** No
    If yes, describe the procedure for sharing data. Include with whom it will be shared, how and why.

## Part C

The attached investigation involves the use of human subjects. I understand the university's policy concerning research involving human subjects and I agree:

1. To obtain voluntary and informed consent of subjects who are to participate in this project.
2. To report to the IRB any unanticipated effects on subjects which become apparent during the course of, or as a result of, the experimentation and the actions taken.
3. To cooperate with members of the committee charged with continuing review of this project.
4. To obtain prior approval from the committee before amending or altering the scope of the project or implementing changes in the approved consent document.
5. To maintain the documentation of consent forms and progress reports as required by institutional policy.
6. To safeguard the confidentiality of research subjects and the data collected when the approved level of research requires it.

Signature* of the Principal Investigator: _____ Date: _____

Figure B 2. Institutional Review Board Approval Letter

INSTITUTIONAL REVIEW BOARD FOR
HUMAN SUBJECTS

April 9, 2007

Aaron Wells
1861 W. 870 N.
Pleasant Grove, UT 84062

Re: The Effects of Low Frequency Ultrasound in Transdermal Drug Delivery

Dear Aaron Wells,

This is to inform you that Brigham Young University's IRB has approved the above research study.

The approval period is from **4/9/2007 to 4/8/2008. Your study number is X07-0097. Please be sure to reference either this number in any correspondence with the IRB.**

Continued approval is conditional upon your compliance with the following requirements:

- A copy of the **Informed Consent Document**, approved as of 4/9/2007, is enclosed. No other consent form should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject must be given a copy of the signed consent form.
- All protocol amendments and changes to approved research must be submitted to the IRB and not be implemented until approved by the IRB.
- A few months before this date we will send out a continuing review form. There will only be two reminders. Please fill this form out in a timely manner to ensure that there is not a lapse in your approval.

If you have any questions, please do not hesitate to call me.

Sincerely,

Dr. Renea L. Beckstrand, Chair /
Nancy A. Davis, CIM, Administrator
Institutional Review Board for Human Subjects
RLB/sm
Enclosures

BRIGHAM YOUNG UNIVERSITY · A-285 ASB · PROVO, UTAH 84602
(801) 422-3841 / FAX: (801) 422-0620

Figure B 3. Institutional Review Board Consent to be a Research Subject _____

**The Effects of Low Frequency Ultrasound in Transdermal Drug Delivery**

Informed Consent to Be a Research Subject

**1. Description and purpose of the research study**

The purpose of this research study is to evaluate the efficacy of transdermal drug delivery when varying the frequency of the ultrasound during phonophoresis. Therapeutic ultrasound is an FDA approved treatment. The practice of combining topical medication with ultrasound gel to deliver medication to tissues is common in therapeutic settings.

Aaron M. Wells, MS, ATC,L is conducting this research study, and he is assisted by Dr. David O. Draper, EdD, ATC,L. Aaron Wells is a certified Athletic Trainer and Doctoral Candidate at Brigham Young University. Dr. Draper is a certified Athletic Trainer and professor of Athletic Training at Brigham Young University.

You have volunteered to participate on your own free will.
You possess a lean Gastrocnemius muscle.

**2. Exclusion Criteria**

Females currently menstruating are asked not to participate. Individuals with calf bruise, infection, open wound, rash, swelling, decreased circulation in the area to be treated, deficits in sensation in the area to be treated, presence of a pacemaker, malignancy, needle phobia, or injury to either lower extremity within the past 2 weeks **should not participate in this study**.

**3. Procedure**

If you choose to participate in this research study on transdermal drug delivery you will be asked to make a visit to the Therapeutic Modalities (RM 123) in the Richards Building at BYU. Prior to beginning the treatment a 5 cm area of both the left and right gastrocnemius muscle will be cleansed with an antibacterial agent (betadine). Two small sterile microdialysis probes will be inserted no greater than 2 cm into each gastrocnemius muscle located proximal to the musculotendinous junction of the achillis tendon. A 90 minute recovery period will be used to allow the body to adjust to the insertion of the probes prior to the application of treatment. You may feel gentle warmth during the ultrasound treatment. The microdialysis probes will collect interstitial fluid during application of the treatment. The total anticipated time at the Therapeutic Modality Laboratory will be 150 minutes.

**4. Risks/Discomforts**

**Risks**

As with the introduction of any instrument through the skin barrier there is a small chance of infection related to participation in this study. However, these research methods have need used with several subjects with 0% incidence of infection.

**Discomforts**

The introduction of the small microdialysis probe into the gastrocnemius muscle will create a discomfort similar to that of having blood drawn during a laboratory test. During the treatment it is possible that you may feel gentle warmth in the arm. However, the treatment at no time should produce a burning sensation or any pain or discomfort. In the unusual instance that pain or discomfort does arise you should immediately inform the researcher and the treatment will be altered or terminated.

**5. Benefits**

There are no known health benefits to you, the subject, in participating in this study. However, this research will help health care professionals to understand optional methods of drug delivery as well as further identify non-thermal affects of ultrasound use.

**6. Confidentiality**

Participation in this research study is voluntary. You have the right to withdraw form this study at anytime, including after supplying informed consent. Withdrawing from this study will not affect your grades or standing as a BYU student or employee. Strict confidentiality regarding your identity and participation in this study will be maintained. No individual identifying information will be disclosed. You will be assigned a subject number and at no time will your name be disclosed during the study or in any manuscripts that may be published from the study.

**7. Researchers names/ phone numbers/ address**

Should you have any questions regarding participation in this study please contact:

Aaron Wells
Brigham Young University
267 Smith Field House
Provo, UT 84602
(801) 422-8674

**8. Information regarding the rights of the research participants**

If you would like additional information on you rights as a research participant in this research project you may contact the Institutional Review Board at Brigham Young University.

**9. Signature**

I have read, understood, and received a copy of the above consent and desire of my own free will to participate in this study and accept the risks relating to this study.

_____
Research Subject's Name (please print)

_____ _____
Research Subject's Signature Date

Figure B 4. Subject Setup Position

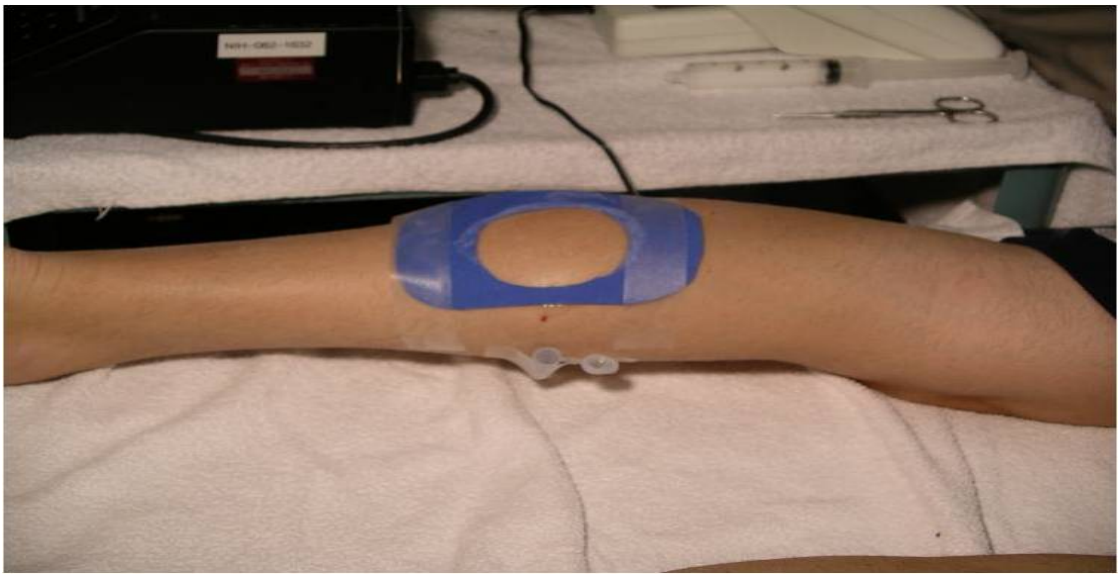
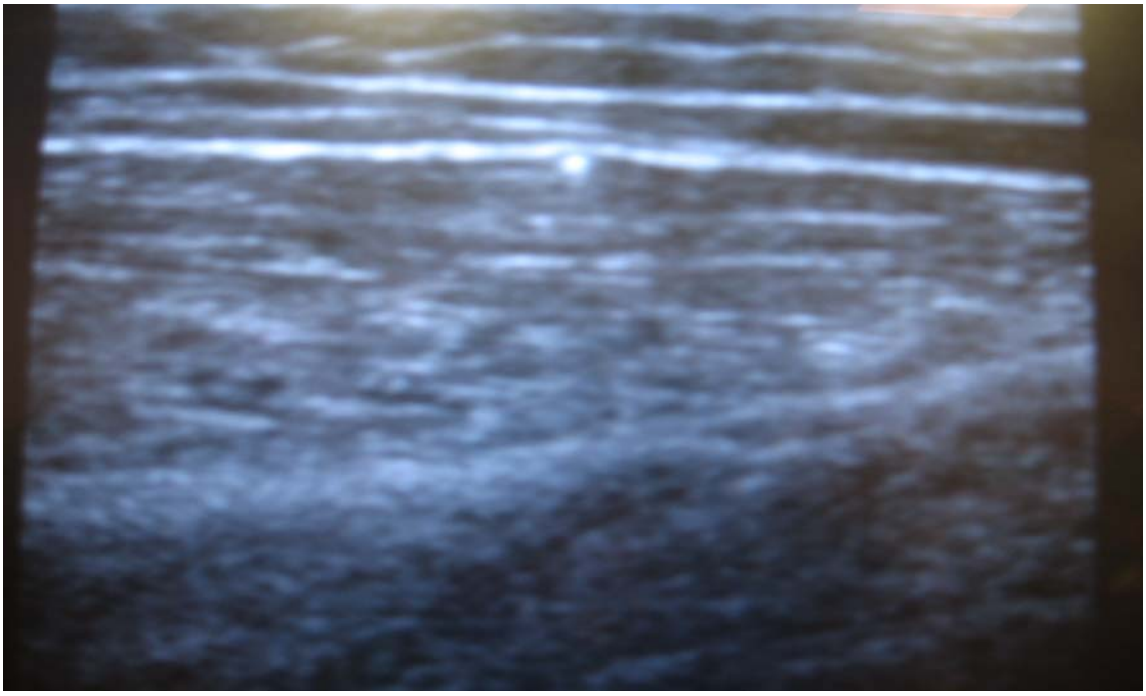


Figure B 5. Doppler Ultrasound Image of Microdialysis Probe



# Appendix C

## Additional Results

Table C 1. Table of Tables of Additional Results

**Table C 2. Sham Group Individual Measurements.**

| Subject | Pre Cortisol (ng/ml) | Post Cortisol (ng/ml) | Change* (ng/ml) | Muscle Depth (cm) | Probe Depth (cm) |
|---|---|---|---|---|---|
| **Control Limb** | | | | | |
| 1 | 11.9 | 29.9 | 18.1 | 0.34 | 1.37 |
| 2 | 12.9 | 11.9 | -0.2 | 0.44 | 1.04 |
| 3 | 11.3 | 11.9 | 0.5 | 0.25 | 0.90 |
| 4 | 23.7 | 19.2 | -4.5 | 0.27 | 1.23 |
| 5 | 14.1 | 15.9 | 1.8 | 0.30 | 1.11 |
| 6 | 11.6 | 16.6 | 4.9 | 0.20 | 1.28 |
| 7 | 12.3 | 13.2 | 0.9 | 0.24 | 1.34 |
| 8 | 10.4 | 13.4 | 3.0 | 0.23 | 1.01 |
| 9 | 20.3 | 15.4 | -4.8 | 0.19 | 0.80 |
| 10 | 14.2 | 11.0 | -3.2 | 0.23 | 1.22 |
| **Average** | **14.2** | **15.8** | **1.7** | **0.27** | **1.13** |
| **Treatment Limb** | | | | | |
| 1 | 13.2 | 10.4 | -2.8 | 0.41 | 1.03 |
| 2 | 11.7 | 12.1 | 0.4 | 0.49 | 1.59 |
| 3 | 15.9 | 34.7 | 18.7 | 0.15 | 1.13 |
| 4 | 18.1 | 18.7 | 0.6 | 0.30 | 1.46 |
| 5 | 16.4 | 16.7 | 0.3 | 0.35 | 1.79 |
| 6 | 13.9 | 14.9 | 0.3 | 0.22 | 1.63 |
| 7 | 15.1 | 18.3 | 3.2 | 0.21 | 1.50 |
| 8 | 12.9 | 17.3 | 4.3 | 0.22 | 1.22 |
| 9 | 16.5 | 13.6 | -2.9 | 0.23 | 1.15 |
| 10 | 28.2 | 17.1 | -11.1 | 0.25 | 1.29 |
| **Average** | **16.2** | **17.3** | **1.1** | **0.28** | **1.38** |

*Change is the difference between pre-treatment cortisol and post treatment cortisol measurement

**Table C 3. 45 KHz Group Individual Measurements.**

| Subject | Pre Cortisol (ng/ml) | Post Cortisol (ng/ml) | Change* (ng/ml) | Muscle Depth (cm) | Probe Depth (cm) |
|---|---|---|---|---|---|
| **Control Limb** | | | | | |
| 1 | 18.9 | 20.3 | 1.3 | 0.26 | 1.45 |
| 2 | 19.7 | 17.4 | -2.3 | 0.27 | 1.15 |
| 3 | 14.1 | 13.0 | -1.0 | 0.24 | 1.18 |
| 4 | 17.3 | 12.5 | -4.8 | 0.31 | 0.78 |
| 5 | 19.9 | 20.0 | 0.2 | 0.25 | 1.65 |
| 6 | 11.6 | 12.6 | 0.9 | 0.34 | 1.17 |
| 7 | 15.5 | 13.4 | -2.2 | 0.25 | 1.09 |
| 8 | 12.4 | 14.6 | 2.2 | 0.26 | 1.28 |
| 9 | 14.5 | 12.0 | -2.5 | 0.29 | 1.79 |
| 10 | 20.2 | 16.8 | -3.3 | 0.25 | 1.27 |
| 11 | 18.5 | 16.1 | -2.4 | 0.22 | 1.18 |
| **Average** | **16.6** | **15.3** | **-1.3** | **0.27** | **1.27** |
| **Treatment Limb** | | | | | |
| 1 | 18.9 | 20.0 | 1.2 | 0.29 | 1.23 |
| 2 | 16.4 | 18.9 | 2.6 | 0.26 | 1.08 |
| 3 | 14.8 | 13.8 | -1.0 | 0.27 | 1.57 |
| 4 | 12.2 | 11.8 | -0.5 | 0.32 | 1.44 |
| 5 | 20.6 | 24.4 | 3.8 | 0.29 | 1.68 |
| 6 | 11.8 | 12.8 | 0.9 | 0.32 | 1.60 |
| 7 | 15.4 | 15.3 | -0.1 | 0.23 | 1.17 |
| 8 | 13.5 | 14.9 | 1.4 | 0.26 | 1.17 |
| 9 | 15.9 | 16.1 | 0.8 | 0.28 | 0.90 |
| 10 | 18.9 | 22.0 | 3.1 | 0.19 | 0.90 |
| 11 | 18.1 | 19.1 | 1.0 | 0.24 | 1.05 |
| **Average** | **16.1** | **17.2** | **1.1** | **0.27** | **1.25** |

***** Change is the difference between pre-treatment cortisol and post treatment cortisol measurement

**Table C 4. 1 MHZ Group Individual Measurements.**

| Subject | Pre Cortisol (ng/ml) | Post Cortisol (ng/ml) | Change* (ng/ml) | Muscle Depth (cm) | Probe Depth (cm) |
|---|---|---|---|---|---|
| **Control Limb** | | | | | |
| 1 | 21.3 | 11.6 | -9.78 | 0.17 | 1.01 |
| 2 | 10.1 | 26.0 | 15.9 | 0.31 | 1.20 |
| 3 | 13.8 | 20.7 | 6.9 | 0.29 | 1.70 |
| 4 | 12.1 | 13.6 | 1.6 | 0.26 | 1.31 |
| 5 | 13.3 | 16.8 | 3.5 | 0.24 | 1.14 |
| 6 | 14.8 | 13.8 | -0.9 | 0.29 | 1.16 |
| 7 | 14.5 | 12.4 | -2.1 | 0.27 | 1.00 |
| 8 | 17.6 | 17.9 | 0.4 | 0.31 | 1.32 |
| 9 | 13.8 | 15.1 | 1.3 | 0.28 | 1.66 |
| 10 | 34.7 | 21.1 | -13.1 | 0.29 | 1.40 |
| **Average** | **16.5** | **16.9** | **.4** | **0.27** | **1.29** |
| **Treatment Limb** | | | | | |
| 1 | 16.8 | 30.1 | 3.3 | 0.22 | 1.14 |
| 2 | 14.1 | 35.4 | 1.3 | 0.30 | 1.31 |
| 3 | 15.7 | 16.1 | .4 | 0.23 | 1.25 |
| 4 | 14.3 | 17.2 | .9 | 0.28 | 1.65 |
| 5 | 12.4 | 19.4 | .9 | 0.24 | 1.22 |
| 6 | 15.6 | 14.2 | 1.4 | 0.27 | 1.20 |
| 7 | 12.8 | 11.6 | 1.3 | 0.30 | 1.54 |
| 8 | 21.1 | 16.5 | 4.6 | 0.30 | 1.28 |
| 9 | 14.2 | 14.7 | .5 | 0.25 | 1.34 |
| 10 | 21.5 | 24.1 | .6 | 0.29 | 1.32 |
| **Average** | **15.9** | **19.9** | **.1** | **0.27** | **1.33** |

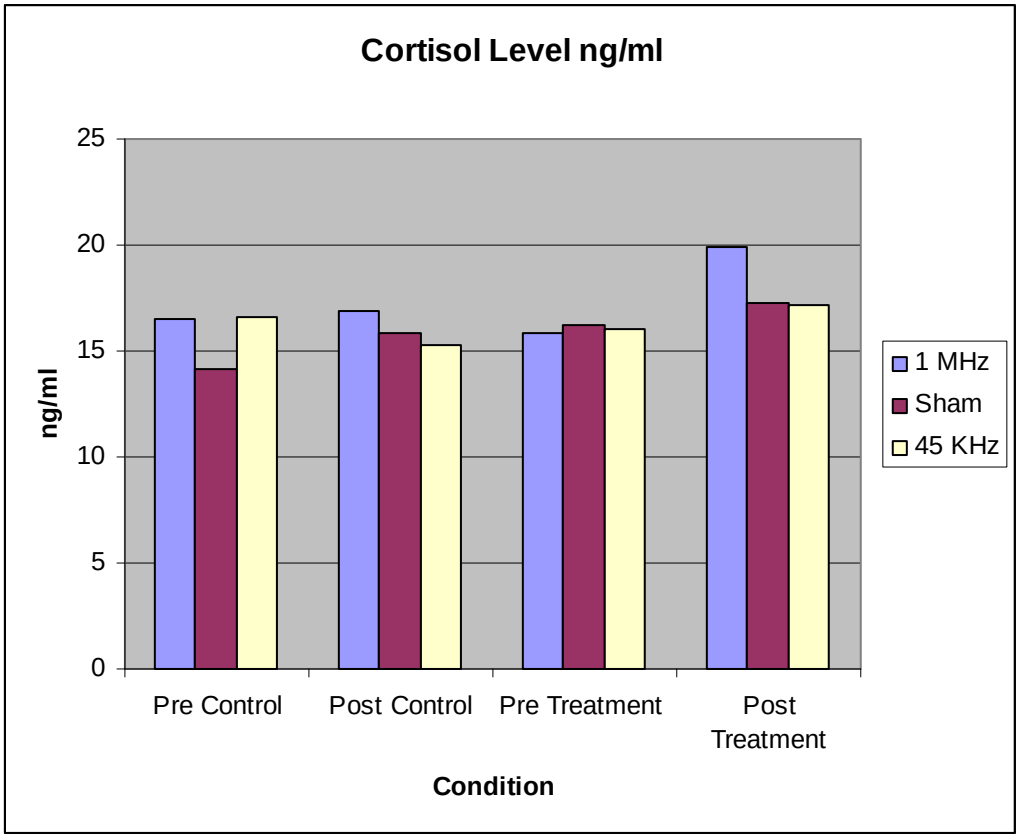
***** Change is the difference between pre-treatment cortisol and post treatment cortisol measurement

**Table C 5. Change* in Dialysate Cortisol Levels (ng/ml); Mean ± SD and Range**

| | Sham | 5 KHz | MHz |
|---|---|---|---|
| **Control** | 1.7 ± 6.6 | 1.3 ± 2.2 | .4 ± 8.1 |
| **Treated** | 1.1 ± 7.5 | 1.1 ± 1.5 | .1 ± 7.8 |
| **Range** | | | |
| **Control** | 18.1 – -4.9 | 0.2– -4.8 | 5.9 – -13.1 |
| **Treated** | 18.7 – -11.1 | 0.8– -0.5 | 21.3 – -4.6 |

* Change is the difference between pre-treatment cortisol and post treatment cortisol measurement

Figure 1. Treatment and Control Dialysate Cortisol Levels (ng/ml)



# Appendix D

## Recommendations for Future Research

**Recommendations for Future Research to Extend the Results of this Dissertation**

1. Conduct testing at the same time each day with all subjects to help control for variation in cortisol levels. Cortisol levels vary throughout the day.
2. Conduct testing in the morning, no more than 3 hours after being awake and no less than 1 hour from sleep. Control activity level by restricting exercise prior to testing. Cortisol levels are generally higher in the morning and fluctuate depending upon activity following waking.
3. Insert all probes into the tissue at a depth of .75 cm. Decrease the attenuation of the ultrasound waves and distance the medication must travel to the collection site to decrease variance.
4. Increase the sample size. Increased sample size permits greater power in stating results.
5. Utilize a drug with a different molecular size, affecting its ability to pass the stratum corneum (dexamethasone, lidocaine). Smaller drug molecules may pass the stratum corneum more easily, thus enabling better evaluation of TDD.
6. Treat each subject's skin prior to treatment with a thorough cleansing to facilitate more equality in the stratum corneum. Treating the skin may increase stratum corneum permeability.
7. Consider performing test with one group receiving an ice treatment prior to application of ultrasound to limit the drug being carried away prior to reaching the target tissues. Reduced blood flow to the target tissue may decrease the drug from being "taken up" superficially into the blood stream once it passes through the stratum corneum layer and reaching exact target tissues.

8. Continue to measure and control for muscle depth, collection depth or probe depth, and pre-cortisol levels. This study has shown they can affect the level of drug delivery, as well as assist in clarifying post – treatment results.