Comparisons of Three Analytical Techniques for Measuring Absolute and Relative Temperature Changes During Ultrasound Treatment

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COMPARISONS OF THREE ANALYTICAL TECHNIQUES FOR MEASURING
ABSOLUTE AND RELATIVE TEMPERATURE CHANGES
DURING ULTRASOUND TREATMENT

by

Michael G. Miller

A Dissertation
Submitted to the
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Advisor: Brooks Applegate, Ph.D.

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Ultrasound is a thermal modality that utilizes acoustic energy to promote heating. While there are many factors that affect heating of body tissues, the effects of skinfold thickness and skin temperature upon ultrasound heating have not been studied extensively. In addition, while temperature typically follows a linear trend, past research typically uses ANOVA or regression analysis to examine this relationship; but these models examine the within-subject effects (time) and between effects (usually groups) at the group level, not the individual level. Therefore, the purposes of this study are to determine if skin temperature and skinfold thickness are predictors for intramuscular tissue temperature changes during an ultrasound treatment and to examine different statistical models for this data. Thirty-two subjects had an absolute intramuscular depth measured at 1.5 cm from the surface of the calf and relative intramuscular depth at one-half the skinfold thickness added to the absolute depth. Two temperature probes were inserted at the respective depths and a surface temperature wire was affixed to the middle one-third of the treatment area. An ultrasound treatment consisting of 1.0 W/cm² using a 3 MHz frequency was applied until the absolute temperature reached 3° above baseline temperature. Data were analyzed with ordinary least squares regression,
hierarchical linear modeling (HLM), and mixed methods repeated measures (MMRM) techniques. Based upon the different trends within the data, it was concluded that HLM, because of examining the within-person effects and parsing out the error components, was a better fit model for analysis. HLM showed that skin temperature was a significant predictor for absolute and relative intramuscular temperatures, while regression MMRM showed skin temperature to be predictive for relative temperature. HLM also showed skinfold thickness to be predictive of relative temperature. Total heating rates for absolute and relative intramuscular depths were 0.61°C and 0.42°C per minute, respectively. In conclusion, it appears that tissue temperatures at the relative intramuscular depth (greater than 1.5 cm) can be better predicted using skin temperature and skinfold thickness and that HLM, examining the within-subject temperature variability during the ultrasound treatment, was the appropriate model.
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Michael G. Miller
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Ultrasound is a thermal modality that utilizes acoustic energy to promote heating. The thermal effects are often used to increase range of motion, metabolic activity, and extensibility of collagen tissue (Draper & Prentice, 2003). However, the temperature required for these therapeutic properties must be accurately known within each person, which is not feasible in the clinical setting. Clinicians who want the desired therapeutic heating effects must rely on specific physiological characteristics based upon temperatures achieved in tissues from past studies. These temperature benchmarks were established in the late 1960s and are as follows: a 1°C increase from baseline temperatures will increase metabolic cellular activity, a 2–3°C increase will decrease muscle spasms and pain and increase blood flow, and a 4°C temperature change will increase collagen extensibility (Lehman, DeLateur, Warren, & Stonebridge, 1967a, 1967b).

The specific rate and change in intramuscular tissue temperatures have been found to vary according to the frequency of the ultrasound treatment and depth of energy penetration. In a study by Draper, Castel, and Castel (1995), the rate of heating was measured at 0.8 and 1.6 cm depth for 3 MHz frequency every 30 seconds during a 10-minute treatment. The rate of heating for 3 MHZ was found to be approximately 0.58°C
per minute based upon mean temperatures per minute over the 10-minute treatment period. Other studies also showed similar heating rates, all based upon mean temperatures (Holcomb & Joyce, 2003; Johns, Straub, & Howard, 2007a; Miller, Longoria, Cheatham, Baker, & Michael, 2008). However, the heating rates are more dependent upon the type of tissue targeted, with more dense or higher collagen content of tissue structures affecting the heating rates (Draper & Prentice, 2003).

The effects of subcutaneous tissue thickness on intramuscular temperatures have been studied over the years. In a study by Lowdon and Moore (1975), deep tissue temperature is inversely related to skinfold thickness and limb circumference, while Johnson, Moore, Moore, and Oliver (1979) reported that a positive relationship exists between body fat and intramuscular temperature changes. Merrick, Jutte, and Smith (2003) showed that different types of cold modalities produced different surface and intramuscular temperature changes. Specifically, using cold modalities, skinfold thickness divided into layers from less than 10 mm to greater than 31 mm showed that the thicker the skinfold, the longer the time to cooling (Otte, Merrick, Ingersoll, & Cordova, 2002).

While skinfold thickness seems to play an important role when determining intramuscular temperatures, all of the aforementioned studies were conducted using cryotherapy. How skinfold thickness affects temperature changes using a deep heating modality such as ultrasound has not been studied extensively in the literature. Ultrasound energy has been postulated to pass through subcutaneous adipose tissue easily without losing energy (Draper & Sunderland, 1993) and that adipose tissue has about one-half the absorption characteristic of muscle (Draper & Prentice, 2003). In a study by Draper and Sunderland (1993), intramuscular temperature probes were inserted 3 cm below skin
surface, then subcutaneous tissue thickness was subtracted to determine actual placement of the probe in the muscle. Ultrasound treatment was applied using 1.0 MHz for 10 total minutes or until temperature did not alter after three consecutive 30-second readings. Based upon the results, temperatures in the muscle were similar for subjects who had a subcutaneous tissue thickness above or below 10 mm, indicating that subcutaneous tissue thickness does not alter ultrasound temperature treatments. However, all subjects had their tissue temperatures raised to a set point with no account for the time required to reach the critical level. Thus, the extent to which adipose affects ultrasound heating remains speculative. In a subsequent published abstract, temperature in the adipose was recorded during an ultrasound treatment and results indicated that the adipose tissue heating was similar to that of the muscle temperature in the same region (Castel, Draper, & Abergel, 1998). These results suggest that adipose may have heating characteristics similar to higher protein content tissues, such as muscle or tendon. What is still speculative is how skinfold affects intramuscular temperature with 3 MHz ultrasound treatments.

The critical temperature values required for therapeutic benefits, described above, have been measured with invasive methods, such as thermocouples (needle temperature probes) inserted into the muscles, not typically used in the clinical setting. The use of thermocouples to measure intramuscular temperature each time a treatment is provided for patients is unrealistic, expensive, painful, and unethical. A noninvasive method that can predict intramuscular temperatures without causing pain or discomfort for patients is needed. Measurement of skin temperature during an ultrasound treatment may potentially be used to predict the heating rate of muscle. Upon review of the ultrasound literature,
skin temperature recordings have not been identified. If skin temperature changes are predictive of intramuscular temperatures while accounting for adipose tissue, clinicians would have a noninvasive method to accurately predict the critical temperatures needed to produce the thermogenic therapeutic effects.

Historical Background of the Problem

Ultrasound is a frequently used modality as a diagnostic agent, to destroy tissues, or, most commonly, as a therapeutic agent for stimulating warmth by alterations of cellular properties. While diagnostic ultrasound is widely used in hospital settings for viewing images of the body, therapeutic ultrasound is often used in sports medicine for injury treatment (Draper & Prentice, 2003). In a survey by Wong, Schumann, Townsend, and Phelps (2007), physical therapists indicated that they used ultrasound for a variety of indications, including decreasing soft tissue inflammation, increasing tissue extensibility, enhancing scar tissue remodeling, decreasing pain, and decreasing swelling.

The inaudible acoustical vibrations of ultrasound can produce either thermal or nonthermal effects in the tissues of the body, depending upon setting parameters. The acoustical energy causes molecular vibrations. As the molecules vibrate, adjacent molecules also vibrate via the acoustical energy, causing the ultrasound “wave” to propagate throughout the area targeted (Haar, 1978). The waves travel either longitudinally or transversely through the tissues. Longitudinal waves displace the molecules in the corresponding direction of the ultrasound transducer head (up and down). In a transverse wave, the molecules are displaced perpendicular to the direction of the wave.
Therapeutic ultrasound has a frequency of above 20 kHz (inaudible), with most ultrasound units varying from a frequency range of .75 MHz (megahertz = 1,000,000 cycles per second) to 3.3 MHz. According to Draper and Prentice (2003), the higher the frequency, the more the ultrasound waves will be absorbed in the superficial tissues, while the lower the frequency, the greater depth of the wave penetration.

As the wave is propagated through tissue, some of the energy will decrease in energy intensity called attenuation. The energy is absorbed, dispersed, and scattered as a result of reflection or refraction (Haar, 1978). Ultrasound energy easily passes through tissues high in water content, but is absorbed in tissues that are denser or have more protein content. Dense tissues, such as muscle, bone, and nerve, where most of the absorption occurs, generate the most heat. The thermal effects of ultrasound increase metabolic activity and blood flow and produce an analgesic effect on nerves (Baker, Robertson, & Duck, 2001) and collagen extensibility (Chan, Myrer, Measom, & Draper, 1998). These heating effects are often used in the clinical setting for reducing pain, relieving muscle spasms, and controlling inflammation. In order to achieve these effects, the tissues targeted must reach a critical temperature (Lehman et al., 1967a, 1967b).

Although the effects of ultrasound as a deep-heating modality could be argued, it is still frequently used by clinicians in the rehabilitation and treatment settings. Warden and McMeeken (2002) found that 87% of the clinicians surveyed felt that even though ultrasound had controversial outcomes with use, ultrasound still has relevance and acceptability in practice. Warden and McMeeken showed that ultrasound was used by approximately 84% of all therapists on a daily basis and accounted for approximately 6% of total income by these clinicians in their practices.
The contribution to evaluation, measurement, and research (EMR) will be centered upon three analysis techniques to predict intramuscular heating effects with ultrasound, based upon the repeated measures data and the independent variables of interest, which, to the author’s knowledge, has never been conducted. The data collected in this study represent a time series design, with time points repeated in half-second intervals throughout the treatment period. Ultrasound heating over a designated treatment period usually increases in a linear trend, and past research typically uses ANOVA (Chan et al., 1998; Draper, Castel, & Castel, 1995; Garrett, Draper, & Knight, 2000; Leonard, Merrick, Ingersoll, & Cordova, 2004) or regression (Draper & Sunderland, 1993; Rose, Draper, Schulthies, & Durrant, 1996) analysis to examine this relationship. These models examine the within-subject effects (time) and between effects (usually groups) at the group level, not the individual level (Wu, 1996).

In a study by Draper and Sunderland (1993), a regression analysis was used to examine the relationship between skinfold thickness and tissue temperature. The skinfold ranges were fairly large, 4mm to 30 mm, with a mean of 12.6 ± 6.1 mm and results showed a small nonsignificant positive correlation ($r = .128$). In addition, the temperatures achieved during the ultrasound treatment were averaged for all individuals ($n = 20$). In another study, 24 subjects underwent ultrasound treatments at various depths and different ultrasound frequencies (Draper, Castel, & Castel, 1995). These authors used a factorial ANOVA to determine mean differences in temperatures over time. Heating rates per minute were based upon average ending temperatures dependent upon
frequency, intensity, and depth across subjects, without examining any within-subject variations. In Holcomb and Joyce (2003), ultrasound temperature was measured by a within/between ANOVA based upon mean ending temperatures of all subjects. In Merrick, Bernard, Devor, and Williams (2003), comparisons of ultrasound devices on intramuscular temperature were examined on six subjects using a MANOVA, adjusting for temperature changes by subtracting the ending temperature from beginning temperature. No mention was made of checking for data assumptions and rationale for selecting the analysis technique used in these studies.

In addition, without explanation of the data collected in these studies, the analysis models used measure the typical linear temperature trend, which may not be the case across all individuals with ultrasound heating. In one study examining transducer heating properties, the authors found that, although the ending peak thermal temperatures of transducer were within .1°C, the heating curve produced was curvilinear (Demchak, Straub, & Johns, 2007). This study supports the notion that ultrasound heating may not follow a linear trend, and combining all subjects into one group for data analysis may produce erroneous results. Finally, results from data that potentially violate the assumption of normality may produce outcomes that could be misinterpreted by practicing clinicians who may apply incorrect ultrasound parameters when treating patients.

The aforementioned studies utilized mean temperature data of subjects without determining within temperature variations, meaning individual growth curves (over time) are not estimated. While standard deviations were reported for each temperature change, in some cases, the standard deviations were almost 1 degree, which can confound the
results of the study since these deviations are quite large for a 2–3 degree change. An exception, however, was Johns et al. (2007b), who used a MANOVA and Levene’s test of homogeneity between types of transducers. This study did focus on the variability of data in order to determine the best model for the analysis.

Therefore, to bring focus to these analytical discrepancies, three analysis techniques—ordinary least squares (OLS) regression, hierarchical linear modeling (HLM), and mixed methods repeated measures (MMRM)—were employed to examine the relationship of the independent variables (skin temperature and skinfold thickness) on the overall degree change for the treatment period. OLS regression was chosen since it is historically a common technique for making predictions of one or two independent variables and is utilized in ultrasound research. HLM was chosen because it estimates individual growth curves and can account for missing data points or varying measurement intervals. MMRM was chosen because of its similarity to HLM, which allows both fixed and random effects, it can handle missing data, and it allows flexibility for specifying the covariance relationship among the measures.

In ultrasound research, regression is used to measure how a particular variable can predict temperature during treatment in order to show how temperature is affected over time. OLS regression techniques are useful not only for controlling confounding variables but also for predicting the “strength of association” of sets of predictor variables or a single variable with the dependent variable (Hoyt, Imel, & Chan, 2008). Regression provides information about not only the overall model used in the analysis, but also the contribution of the individual predictor variables within the overall model. Regression is based upon the premise that errors have a finite variance, linearity, and homoscedasticity.
This can be measured, among other ways, by residual scatter plots. The residual scatter plots should show how the data points are scattered in order to determine a visual representation of the normality. Linearity of the residuals, if not met, weakens the power of the regression. For linearity, there should be a straight line relationship with the predicted values.

However, OLS regression uses means scores, typically at the end of the treatment instead of examining all data points from beginning to end to form a regression equation. An adequate sample size is also needed to strengthen the relationship between the variables of interest. The sample size recommended, according to Tabachnick and Fidell (2001), should be 50 plus $8n$, where $n$ equals the number of independent variables. In this study, with two independent variables, theoretically the sample size would need to be 66 subjects, which would not be feasible for the methods of data collection, but with repeated measures where there is less variability within persons over consecutive data points, the total $n$ can be much less.

Hierarchical linear modeling (HLM) is designed to examine longitudinal data and trends associated at the individual level and then to examine how these trends are affected by individual characteristics (Wu, 1996). HLM produces valid estimates when the time points are not equal or missing data are present. HLM uses the repeated measures units (in this study, the units of time are nested within individuals) to develop a growth rate as a level 1 analysis, then uses the estimated parameters in the level 1 model to determine changes within a level 2 model. In other words, individual growth rates are determined, and then these growth trajectories are used to determine between-individual factors (Bryk & Raudenbush, 1987). HLM therefore can examine each subject’s trends, the overall
group's trend or trajectory, and the variance of the individual around the mean of the trend, and can predict the individual's differences in the trends (Hernandez-Lloreda, Colmenares, & Arias, 2003).

Similar to HLM, MMRM essentially compares the within and between effects over time and produces a model equation to interpret how the data can vary both within and between persons. MMRM, in this dissertation, collapsed the within-subject time into a grouping effect, then examined how the level 2 group variable impacted the mean response of the level 1 effect.

Purposes

Clinically, ultrasound is administered for specific lengths of time based upon calculations derived for therapeutic temperature outcomes. While ultrasound can heat tissue, the extent to which subcutaneous tissue thickness (skinfold thickness) affects temperature with 3 MHz ultrasound treatments is still unknown. To determine skinfold thickness (subcutaneous adipose) for subjects and to determine the overall effect of heating tissues with 3MHz ultrasound treatments, specific depth measurements were taken. The first measurement was an absolute measurement from the surface of the treatment area. The absolute measurement, determined based upon the heating effects for 3 MHz ultrasound, was 1.5 cm. A relative depth was calculated to account for the overall skinfold thickness by adding the $\frac{1}{2}$ of the skinfold thickness to the absolute depth, to ensure that the same amount of muscle tissue is exposed to the ultrasound energy, regardless of subcutaneous thickness.
Therefore, the purposes of this study were to examine the following: (a) to determine the overall absolute and relative intramuscular and skin heating temperatures and heating rates, (b) to determine if skin temperature can be used to predict intramuscular temperature, (c) to determine if skinfold thickness affects the heating of intramuscular tissues using therapeutic 3 MHz ultrasound, and (d) to compare the results for all three analytical methods (OLS regression, HLM, and MMRM) on the variables of interest.

If the study shows that skin temperature can predict intramuscular temperature, clinicians can use noninvasive procedures for determining when a critical temperature in the muscles can be achieved, potentially using a prediction equation to determine intramuscular tissue temperature. In addition, if subcutaneous tissue thickness alters ultrasound heating characteristics, more in-depth studies will be needed to determine the relationship of adipose thickness during an ultrasound treatment. Finally, comparison of data analysis models might lead future researchers to incorporate a more appropriate analytical technique for ultrasound treatments.

Research Questions

RQ1: What are the absolute and relative intramuscular and skin temperatures and overall rate of heating per minute for each, respectively, during 3 MHz ultrasound treatment?

RQ2: Does skin temperature predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?
RQ3: Does subcutaneous skinfold thickness predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?

RQ4: Do the three analysis models (OLS regression, HLM, or MMRM) produce similar results for the research questions?

Limitations

The following are limitations inherent in this research. To begin, the researcher stopped ultrasound treatment at a 3°C temperature change from baseline temperature. The subjects volunteering were young and relatively lean in nature, and thus may not provide a range of subcutaneous thickness that may be present with the population as a whole.

Delimitations

The thermocouples may not have been inserted thermocouples exactly at the same depth for the absolute measurement site, thus the depth could vary between persons. Only the calf muscle (triceps surae) was used for measuring temperature differences for the study, since insertion of probes appears to be less painful for subjects in that region. Finally, the researcher used the Omnisound 3000 as the ultrasound unit.

Definition of Terms

*Acoustical Impedance* – changes in the structural properties of tissues that cause the ultrasound wave to reflect or refract.

*Acoustical Streaming* – unidirectional flow currents in a fluid that are due to the presence of sound waves.
**Attenuation** – decrease in energy property as the distance from the transducer increases as a result of absorption or scattering of the energy beam.

**Beam Non-Uniformity Ratio (BNR)** – expresses the ratio of ultrasonic energy delivered to the tissue in terms of overall peak and overall average energy. The higher the ratio, the worse the BNR. Most ultrasound units have a BNR of 4 or 5:1.

**Cavitation** – formation of gas bubbles in a liquid as pressure rises or falls.

**Cryotherapy** – using a cold modality to alleviate pain and reduce inflammation.

**Eddies** – circular motion of a fluid that moves against the main flow.

**Effective Radiating Area (ERA)** – the area of the ultrasound head that produces the ultrasonic waves.

**Indirect Piezoelectric Effect** – the conversion of electrical energy into a form of mechanical energy.

**Megahertz (MHz)** – equates to 1,000,000 hertz.

**Near Zone** – ultrasound energy located in the tissues directly underneath the ultrasound transducer head.

**Skinfold** – a measurement of subcutaneous body fat by pinching the skin site.

**Spatial-Average Intensity** – measured in Watts/cm², it describes the amount of power per until area of the ultrasound transducer effective radiating area.

**Spatial-Peak Intensity** – the maximum output of the ultrasound energy across the entire effective radiating area.

**Temporal Peak Intensity** – the maximum amount or intensity of the ultrasound wave found only during the “on” time.
Temporal-Average Intensity – the averaged amount of ultrasound energy during both the “on” and “off” times.

Thermocouples – a temperature sensor that produces a voltage in response to differences in thermal gradients.

Watts – a unit of power that represents 1 joule of energy per second.
CHAPTER II

REVIEW OF LITERATURE

The purposes of this chapter are to review the relevant literature that pertains to therapeutic ultrasound and the three analytical techniques. This chapter is divided into sections, based upon ultrasound characteristics, parameters, and analysis techniques. First, a review of ultrasound theory (energy) will be discussed. Second, a review ultrasound unit characteristics and physiological characteristics of the thermal effects when applying ultrasound will be discussed. The third part of the chapter will review tissue characteristics, in particular subcutaneous and skin temperature that can affect ultrasound energy transmission. Ultrasound unit variability along with coupling mediums and how they affect the transmission of energy or production of thermal effects will be reviewed. Clinician use of ultrasound for injuries and pathologies, along with perceived benefits, will complete the ultrasound topic. Finally, a review of the statistical analysis models that contribute to the evaluation, measurement, and research field will be provided in context to this dissertation.

Ultrasound Energy

Ultrasound energy is transmitted via an ultrasound unit generator. The ultrasound unit generator consists of the main unit where selection of frequencies and intensity can be manipulated and a transducer head or ultrasound head that delivers the ultrasound
energy. The main unit generator transmits electricity via a coaxial cable to the transducer head. Inside the transducer head, a quartz or synthetic ceramic crystal that is 2–3 mm thick will change shape when a voltage is applied to it via electrical energy (Draper & Prentice, 2003; Haar, 1978). The voltage applied from electrical current causes the crystal to compress and expand (vibrate) at the set frequency. The process of applying an electrical energy to a crystal to produce acoustical energy is called an indirect or reverse piezoelectric effect.

Ultrasound energy is mechanical energy, consisting of sounds wave of frequencies greater than 16 kHz, which are above any audible range for humans (Haar, 1978). In therapeutic ultrasound, frequencies typically range between 1 and 3.3 MHz with 1 MHz equal to 1,000,000 cycles per second. The higher the frequency, the more likely the sound energy will diverge and spread out. Thus, high frequency ultrasound (3 to 3.3 MHz) is better suited for superficial treatment sites and lower frequencies are best for deeper tissue treatments. The velocity of transmission of ultrasound is dependent upon the density of the tissue (the protein content of the tissue structures) (Draper & Prentice, 2003). Tissues with a higher density tend to allow higher molecular velocities, that is, as the sound waves hit the denser materials, there is more movement and faster rate of molecular velocity of the sound energy through that tissue.

As ultrasound energy propagates into tissues, the sound wave travels in longitudinal and transverse directions. Longitudinal waves displace molecules in the direction in which the wave travels down the transducer head. Transverse waves move molecules in a perpendicular direction. Transverse waves occur when the energy hits molecules and the vibrations hit adjacent molecules, thus propagating movement in the
transverse direction. Both longitudinal and transverse molecular movements occur with ultrasound energy, but the magnitudes of transverse waves are usually much less than ones that are longitudinally propagated. Movements of the waves are dependent upon the movement of cellular particles that oscillate in the direction of the wave (Haar, 1978). In ultrasound waves, areas of high molecular density in the tissues are called compressions, while areas of lower molecular density are called rarefactions (Draper & Prentice, 2003).

Sound waves will decrease in energy intensity as they travel through tissue, called “attenuation.” Attenuation occurs due to absorption, dispersion, or scattering of the sound wave. Tissues high in water content allow penetration of the sound energy through it, whereas energy is absorbed in tissues with higher density. The denser the tissue, the more heat is produced due to molecular vibrations and friction (Haar, 1978). Ultrasound energy will also reflect or refract when interfacing different tissue boundaries, such as between soft tissue and adipose or between soft tissue and bone. This phenomenon is often referred to as “acoustic impedance.” The greater the difference between two interfering surfaces, the more reflection or scattering of the ultrasound wave. When the energy hits higher tissue impedance, the reflected energy then combines with the transmitted energy coming from the ultrasound transducer, creating an area of higher energy potential called a standing wave or “hot spot” that can cause tissue damage or increased heat production.

The ultrasound energy beam is dependent upon the size of the sound head or transducer. The larger the transducer head, the more collimated (focusing) the energy beam becomes. Smaller transducers produce a more divergent ultrasound energy beam (Draper & Prentice, 2003, p. 104). In addition, 1 MHz has more of a divergent beam compared to 3 MHz ultrasound frequency. Within the beam itself, the energy is not
always distributed evenly. In the near field or near zone (area closest to the transducer head), the energy fluctuates because of pressure differences from the creation of the energy wave of the transducer. In the far field or far zone, the energy distribution is more uniform but the beam is more divergent. The energy beam is often measured by the beam nonuniformity ratio (BNR). The BNR is determined by measuring the maximal intensity of the transducer energy to the average intensity across the transducer surface. The lower the ratio, the more uniform the ultrasound energy beam being delivered to the tissues. The BNR should fall between 2:1 and 6:1 to ensure the uniformity of the ultrasound beam.

The actual portion of the transducer head that produces the sound energy is called the effective radiation area (ERA). The ERA is based upon the size of the crystal found within the transducer head that vibrates and produces the ultrasonic energy. The energy output is measured 5 mm from the surface of the transducer head (Draper & Prentice, 2003). The crystal is always slightly smaller than the transducer and must be taken into account for the actual ERA on a treatment site. As such, the treatment site should be roughly 2–3 times the ERA. If the ERA is larger, the ultrasound energy dissipates quicker over the increased treatment surface area, resulting in non-uniform thermal effects.

The ultrasound energy can be delivered continuously or pulsed. Continuous ultrasound is a constant energy beam being on for 100% of the time during the treatment. With pulsed ultrasound, the energy is intermittent and has periods of no energy being delivered. The amount of energy (pulse duration of the ultrasound energy) delivered with pulsed ultrasound is dependent upon the duty cycle. The duty cycle can be calculated by the “on time” divided by the “on time plus off time” multiplied by 100. Most ultrasound unit generators have duty cycles ranging from 20–50% for pulsed ultrasound.
Finally, intensity of the ultrasound is measured by Watts or Watts divided by the squared size of the transducer head that delivers the ultrasound energy, defined as W/cm². Intensities range from .5 to 3.0 W/cm² in .5 increments. The higher the intensity, the more energy being delivered within the treatment area, which results in faster heating of the tissues. Usually, treatment intensities range from .5 to 1.5 W/cm² because these ranges were found to be more comfortable for patients.

In review, ultrasound energy is based upon several factors. The frequency of vibrations (MHz) determines the actual penetration within tissues. As the frequency decreases, the depth of penetration increases. In addition, the frequency contributes to the absorption or reflection of sound waves, causing movement of molecules in longitudinal or transverse directions that are dependent upon the structural component of the tissue targeted. The transducer size and the crystal inside that produces the ultrasound energy are important in determining the ERA. Intensity is also based upon the size of the transducer and the amount of energy delivered within the ERA.

**Transducer Variability on Energy Delivery**

Ultrasound units are made by different manufacturers. Although standardized ultrasonic fields of energy are transmitted with the ultrasound units, the transducers (ultrasound heads) vary in their energy transmission. The variability of the transducer head may contribute to differences in energy output over the treatment ERA (Johns et al., 2007b). In terms of specifying dosages by the spatial average intensity (SAI, which is equal to the output power [Watts] divided by the ERA), a cohort of transducer heads (7, 5 cm²) was selected from one manufacturer (Chattanooga Intellect) to determine if the
ERA, total power, BNR have similar outputs. The transducer heads were placed in degassed water. Outputs were measured by a hydrophone in volts, then converted to pressure, then to intensity. According to the results using a MANOVA, all 7 transducers met the reported manufacturer's specifications for ERA at 1 and 3.3 MHz. Total power at 1 and 3.3 MHz fell within calibration standards. Although the SAI was not significantly different, the range of variability within 1.0 MHz was 17% and up to 50% for 3.3 MHz with the transducer heads. These results suggested that a variability range in SAI between transducer heads, although within manufactured reported guidelines, may contribute to differences in tissue heating parameters between transducers of various ultrasound units, even from the same manufacturer.

Similarly, another study examined differences in transducers of the same manufacturer to determine heating variability existence (Demchak et al., 2007). In this study, 12 subjects participated in three separate treatments for three difference transducers (counterbalanced design), using the same ultrasound parameters of 1 MHz, at 1.2 W/cm² for 10 minutes with the Omnisound 3000. Prior to the intervention, the three transducers were tested for total power and determined that the SAI was as follows: Transducer A = 1.2 W/cm², transducer B = 1.3 W/cm², and transducer C = 1.4 W/cm² when the Omnisound unit was set to 1.2 W/cm². The subjects had thermocouples inserted into their left calf at a depth of 3 cm plus ½ skinfold thickness. Using an ANOVA, results indicated that there were no significant main effects for transducer but a main effect for time, with all three transducers increasing temperature from baseline to the end of the treatment. The temperature heating, however, was curvilinear and inconsistent between the transducers. There were two distinct phases for transducers A and B, and three for transducer C.
Results indicated that, while all three transducers had similar heating final temperatures, the rate at which the final heating temperature was achieved differed between transducers from the same manufacturer.

The amount of energy delivered to the tissue may also be attributed to the rate or speed of movements of the transducer over the treatment area. While many researchers default to a rate of about 2 to 4 cm/s over the treatment area of 2–3 ERA, rates faster are often applied in the clinical setting. These faster rates may limit the amount of energy absorbed based upon the quickness of movements and lack of time over one area. As such, a study was conducted to determine if differences in transducer rates of 2–3, 4–5, and 7–8 cm/s altered the intramuscular temperature during a 1 MHz ultrasound treatment (Weaver, Demchak, Stone, Brucker, & Burr, 2006). Eleven subjects had a thermocouple inserted at a depth of 3 cm plus ⅔ skinfold thickness in the left calf muscle. An ultrasound treatment was delivered over a 2 ERA template using an intensity of 1.5 W/cm² for 10 minutes. Rate of movement followed the beat of a metronome at 60, 88, and 192 beats per minute calculated by the ERA to correspond to the 2–3, 4–5, and 7–8 cm/s rates. Using a 2 x 3 repeated measures ANOVA, results showed a significant main effect for time with posttreatment values higher than pretreatment values and no significant main effect for velocity and no significant interaction. As a result, the rate of movement dispels the notion that energy is absorbed less with faster rates within the ERA.

In summary, transducers, although manufactured to fall within specific guidelines, differ in energy output. Studies have confirmed that while the ranges of energy output still meet the specific guidelines and criteria, there are some disparities that may occur and
may affect the heating attributes in tissues during ultrasound treatments. However, the rate of transducer movement within the ERA does not appear to make a significant contribution to the heating effect of an ultrasound treatment.

Ultrasound Thermal Effects Characteristics

Ultrasound energy applied to tissues causes several physiological effects that fall within two predominant classifications, nonthermal and thermal. Nonthermal ultrasound causes tissue alterations (increases in cellular permeability) as a result of mechanical effects without any thermal properties. Thermal ultrasound results in increases of tissue temperature from constant molecular vibrations, which do not happen with nonthermal ultrasound. Nonthermal ultrasound is used for acute injuries when temperature increases are not warranted due to the inflammation process. Nonthermal ultrasound is purported to accelerate healing by stimulating phagocytosis in the treatment area, increase free radical production of cells, alter cell permeability, and possibly enhance fibrinolysis (Johns, 2002). Other nonthermal effects include increased cell diffusion rates, protein synthesis, edema reduction, and tissue regeneration (Dyson, 1987; Dyson & Pond, 1970).

Tissue alterations from nonthermal ultrasound are caused by either acoustical microstreaming or cavitation. Acoustical microstreaming is the unidirectional movement of the ultrasound wave in fluids (tissues). The ultrasound energy contacts cell membranes, causing the tissue fluids to flow around the membrane, referred to as eddies. The effects of acoustical streaming causes ions and molecules to displace and alter the cell permeability as it moves around the cell, resulting in movement of calcium into the
cell and potassium and other metabolites into and out of the cell (Johns, 2002). Cavitation occurs as a result of the pressure changes of the ultrasound energy, creating gas bubbles to oscillate in a systematic manner in cellular fluids.

Nonthermal ultrasound effects are produced with pulsed or discontinuous ultrasound. Pulsed ultrasound is usually delivered with a percentage of continuous output, (approximately around 20%). This means that during a treatment, the energy is being delivered only 20% of the time. The less time the energy beam is delivered, the fewer heating effects are produced while still producing cavitation or acoustical microstreaming (Dyson, 1987).

Thermal effects of ultrasound produce heating in the tissues via molecular vibrations. As the ultrasound energy penetrates the tissues, the ultrasound energy beam produces friction or movements of the molecules. Heating of the tissues is beneficial for reducing pain, decreasing or relieving muscle spasms, decreasing muscle contractures, promoting healing, increasing sensory and motor nerve conduction velocity, increasing tissue elasticity, and increasing blood flow (Cole & Eagleston, 1994). According to Starkey (2004), Draper (1998), and Draper, Castel, and Castel (1995), within the ERA, a 1°C increase in tissue temperature from baseline will accelerate the metabolic rate of cells about 13%. A 2–3°C increase in tissue temperature above baseline will decrease muscle spasm and pain, increase blood flow and reduce chronic inflammation. Finally, a 3–4°C temperature increase from baseline will elongate tissue and reduce scar formation.

The heating of the tissue is also dependent upon the frequency and intensity. With 1 MHz at an intensity of 1 W/cm², the muscle heats at a rate approximately .2°C per minute. For 3 MHz at an intensity of 1 W/cm², the rate is approximately .6°C per minute
(Draper, 1998; Draper, Castel, & Castel, 1995). These rates were based upon repeated measures ANOVAs and also based upon averaged ending time and temperature changes and at different depths. Since the intensity of the treatment can be varied, heating rates can vary. The specific heating rates and the frequency used are also dependent upon the depth of the tissue and structure of the tissue and will be discussed in the next section.

In review, ultrasound energy is divided into nonthermal and thermal effects. Nonthermal effects produce cavitation, which is compression and expansion of gas-filled bubbles and acoustical microstreaming is the movement of fluids around a cell. Thermal effects produce tissue heating by movement or friction of molecules during ultrasound energy propagation. The overall rate of heating is also dependent upon the frequency and the intensity of the ultrasound treatment.

Ultrasound Heating Physiological Characteristics

As previously mentioned, temperature increases are beneficial for biophysical events in the body. However, the methods and parameters to produce the heating effects vary for type of tissue, ultrasound frequencies and parameters, and length of time at a critical temperature. Of particular importance is the type of tissue in which temperature changes are sought. While almost all ultrasound studies investigated tissue changes in the calf muscle, some studies investigated temperature changes in other tissue types. Using the right patella tendon, the rate of temperature changes using two different treatment sizes (ERA) was investigated (Chan et al., 1998). The ERA selected for the study were either 2 or 4 ERA using a 4.5 cm² ultrasound transducer (Omnisound 3000) set at 3 MHz of 1.0 W/cm² intensity for a 4-minute treatment. There were 16 subjects who were
randomly grouped with 8 receiving a treatment with a 2 ERA while the other 8 received the 4 ERA treatment. A 2 cm, 26 gauge thermistor was inserted in the mid patella tendon at 1 cm below the apex of the patella. Using a two-factor repeated measures analysis (heating and cooling) and paired t tests for ERA differences, the results indicated that the 2 ERA had significantly higher temperatures (8.3°C ± 1.7) compared to the 4 ERA (5.0°C ± 1.0) treatment site during the treatment period. These results suggest that tissues with higher protein density absorb more energy and have higher increases in temperature. In addition, results showed that the smaller the ERA, the higher the temperature achieved and the longer temperature decay after treatment.

The size of the treatment area and intensities are often manipulated to determine heating of tissues. Temperature changes within the triceps surae (calf) were compared between ultrasound and pulsed shortwave diathermy over a larger ERA (Garrett et al., 2000). Sixteen subjects received either a diathermy treatment first followed by an ultrasound treatment or vice versa. The ultrasound parameters were 1 MHz, 1.5 W/cm² for 20 minutes over a 40 ERA. The diathermy settings were 800 bursts per second with a 400 microsecond burst duration, an 850 microsecond interburst interval with a peak root mean square amplitude of 150 W per burst, or an average root mean square of 48 W per burst over a 20-minute treatment (diathermy measurements, which will not be discussed in this dissertation). Temperature was recorded every minute. Using a two-way ANOVA with repeated measures, results indicated that the diathermy unit heated the muscle significantly more than the ultrasound treatment over the three sites selected in the triceps surae. Results also demonstrated that while using ultrasound over an extremely large ERA, the heating decreases, supporting the notion that ultrasound treatments need to be
confined to a smaller ERA. These results suggest that if large muscles or tissues are targeted for clinical treatment, diathermy units may be more beneficial than ultrasound.

Another study was conducted to determine if the temperature elevation above baseline had a direct physiological effect when the intensity was altered during the treatment (Burr, Demchak, Cordova, Ingersoll, & Stone, 2004). The rationale for this study was based upon the theory that when the tissue reaches a critical set point temperature, the intensity of the ultrasound treatment must be lowered to maintain that temperature. In their study, Burr et al. (2004) measured tissue elevation of 3°C at 3 cm below adipose layer, then maintained that temperature. Twenty subjects underwent two separate ultrasound treatments, one at 2.4 W/cm² for 2.5 minutes, followed by a 7.5-minute treatment at 1.0 W/cm² and a 1.5 W/cm² treatment for 10 minutes using a 1 MHz frequency with the Omnisound 3000. A multivariate analysis to test differences in the ultrasound intensity showed that the 2.4 W/cm² group had a significant increase in temperature at the 2.5 minute time period versus the other group, most likely due to the intensity differences. In terms of tissue temperature elevation over time, there was no significant differences between the groups with the 2.4 W/cm² at 4.9 minutes and the 1.5 W/cm² at 4 minutes, respectively.

In a similar study, intensities levels were compared during a 10-minute ultrasound treatment to determine intramuscular temperature differences (Leonard et al., 2004). Nineteen subjects underwent four intensity level ultrasound treatments—.5, 1.0, 1.5, and 2.0 W/cm²—for 10 minutes at 1 MHz with a Rich-Mar Theratouch 7.7 ultrasound unit. Intramuscular temperatures were measured in the calf at 4 cm below the treatment surface. Using a repeated measures ANOVA for intensity levels, results indicated
significant differences among the four intensities with the 1.0 W/cm$^2$ (37.3°C) and 2.0 W/cm$^2$ (36.1°C) being different. Results also found temperature changes at the respective intensities dissimilar to temperature changes compared to Draper, Castel, and Castel (1995). Their findings also suggest that the values obtained are subjected only to the particular ultrasound unit (Rich-mar) used in study.

The frequency of the ultrasound treatment is another factor that is used for determining temperature changes. For clinicians to adequately determine if temperature changes for the desired temperature have been met, heating rates for the different frequencies of ultrasound must be known. In a study by Draper, Castel, and Castel (1995), 24 subjects, randomly divided into two groups (1 or 3 MHz), underwent ultrasound treatments with an Omnisound 3000 in the left triceps surae with varying intensities—.5, 1.0, 1.5, and 2.0 W/cm$^2$—for 10 minutes or until discomfort. The ultrasound transducer was 5 cm$^2$ with an ERA of 4.1 cm$^2$. Temperature was recorded using a 23 gauge thermistor placed into the center of the treatment area at depths of .8 and 1.6 cm for the 3 MHz treatment and 2.5 and 5 cm depth for the 1 MHz treatment, respectively. Analysis was conducted using a three-factor ANOVA for mean temperature. The rate of temperature rise for 1 MHz at.5 W/cm$^2$ was .4°C, for 1.0 W/cm$^2$ it was .16°C, for 1.5 W/cm$^2$ it was .33°C, and for 2.0 W/cm$^2$ it was .38°C per minute at 2.5 cm depth. Of note, it took more than 10 minutes on average to increase the temperature to 4°C at these parameters, regardless of tissue depth. For the 3 MHz frequency, the rates per minute of tissue temperature increases were the same for both .8 and 1.6 cm and were .3°C for .5 W/cm$^2$, .58°C for 1.0 W/cm$^2$, .89°C for 1.5 W/cm$^2$, and 1.4°C for 2.0 W/cm$^2$. These results suggest that there were no significant differences between depths within each
frequency. The results also produced the heating formulas used by clinicians for desired heating effects during ultrasound treatments. In addition, this study also confirmed that the longer the treatment, the higher the temperatures achieved, and the higher the intensity, the greater the amount of heating.

As previously discussed, the lower the frequency, the deeper the tissue penetration, particularly for thermal effects, with 1 MHz penetrating from 2.5 to 5 cm depth, while 3 MHz penetrates from the skin to 2.5 cm in depth. However, the half-value (half of the purported depths of each frequency), which corresponds to 2.3 cm for 1 MHz and .8-1.6 cm for 3 MHz (Draper, Castel, & Castel, 1995), appears to heat more consistently. To determine the accuracy of these values, a study by Hayes, Merrick, Sandrey, and Cordova (2004) examined the depth penetration of ultrasound, speculating that it may heat deeper than the half value frequently used and may, more specifically, be able to heat to the end depth range more effectively than thought. In their study, they measured temperature in the left calf at 2.5 cm depth and used both 1 and 3 MHz and sham ultrasound treatments (ultrasound not turned on). Each treatment was set at 1.5 W/cm² and continued until the temperature reached 40°C or for 10 total minutes. A repeated measures design analysis indicated that the 3 MHz treatment reached the critical temperature and reached that temperature in an average of 4.13 minutes or at a rate of 1.19°C per minute. While the half-value is thought to be the depth of most energy heating for 3 MHz (at 1.6 cm), the results showed that at 2.5 cm depth, the critical temperature can still be achieved, in almost the same heating rate as the half-value rate.

In a cadaver model, ultrasound frequencies of 1 and 3 MHz for both pulsed and continuous modes at .5, 1.0, 1.5 and 2.0 W/cm² were investigated (Cambier et al., 2001).
Using the same cadaver, a total of 16 experiments were conducted with three temperature probes placed into the left calf at 1, 3, and 5 cm below the surface. An ERA of 5 cm, with the aforementioned intensities and frequencies, were delivered for 10 minutes, respectively, one treatment per day. Results indicated that continuous ultrasound had a higher and greater temperature increases for each intensity. In addition, the lower the frequency, the greater the temperature reached during the treatment. Heating of the tissue also decreased as a function of depth, going from 1.49°C per minute at 1 cm to .09°C per minute at 5 cm using 1 MHz frequencies. These results confirmed the need for further investigations of temperature changes with depth of tissues in human tissues versus cadaver experiments. The specific statistical analyses for these results, however, were not provided.

While ultrasound is predominantly used for heating tissue and treating many tissue pathologies, the effects of concomitant treatments with ultrasound and other therapeutic modalities remain questionable as to the additive or negative effects of both modality treatments on heating tissues. Rimington, Draper, Durrant, and Fellingham (1994) examined the effects of pre-cooling the calf prior to an ultrasound treatment. It was hypothesized that pre-cooling the tissue would slow down molecular movement and make the tissue denser by decreasing the molecular distance. In their study, 16 male subjects received either an ultrasound treatment consisting of 1 MHz at 1.5 W/cm² for 10 minutes or the same ultrasound treatment parameters following application of a 15-minute treatment of an ice bag. Temperature was measured using thermistors inserted at a depth of 3 cm and recorded every 30 seconds. Using an independent t test, results indicated that the ultrasound group increased approximately 2°C, while the pre-cooled
group had a decrease of approximately 4.5°C. Of note, the first 2 minutes of the ultrasound treatment for the pre-cooled group had muscle temperatures that actually decreased, demonstrating that the effects of ice on muscle tissue do not immediately change and increase after application of a heating modality. While the tissues did not heat during the ultrasound treatment, the length of time to pre-cool the area should be decreased to determine if the density of tissues as a result of vasoconstriction may alter the heating parameters of ultrasound.

In a follow-up study by Draper, Schulthies, Sorvisto, and Hautala (1995), the effects of applying an ultrasound treatment following an ice bag treatment for a shorter duration and greater depth were studied. Subjects were randomly assigned into two groups, an ultrasound group and pre-cooled tissue followed by ultrasound group. The ultrasound parameters were 1 MHz at 1.5 W/cm² for 10 minutes using an Omnisound 3000 for both groups. For the pre-cooled group, an ice bag was applied to the left triceps surae for 5 minutes prior to the ultrasound treatment. Temperature was measured at a 5 cm depth from the surface of the treatment area. Using a one-way ANOVA, results indicated that the ultrasound group increased temperature by 4.0 ± .83°C above baseline, while the pre-cooled group increased temperature by 1.8 ± 1.0°C. The ice group had lower initial baseline temperature values prior to the initiation of the ultrasound treatment and may have caused the relatively little changes in temperature elevation.

Conversely, the addition of superficial heat prior to ultrasound treatment may increase the thermal effects. In a study by Draper et al. (1998), the effects of applying a hot-pack to the treatment area prior to ultrasound were examined. In their study, 21 subjects had thermistors inserted at 1 and 3 cm below the calf surface and were randomly
assigned into either a hot-pack and ultrasound group or room-temperature pack and ultrasound. The hot-pack or room-temperature pack was applied for 15 minutes prior to ultrasound treatment of 1 MHz, 1.5 W/cm² for 10 minutes, respectively. Using a 2 × 2 × 3 ANOVA, the hot-pack and ultrasound treatments increased temperature significantly at both depth levels compared to the room temperature and ultrasound group. In addition, it was observed that temperatures in the tissues can reach the critical temperature of 4°C, 2–3 minutes quicker with hot-pack application.

Heating of tissue with ultrasound is based upon many parameters, especially within clinical practices. To further explore the intramuscular temperature changes, a study was conducted to determine the observed clinical parameters versus the recommended research parameters for heating tissues (Demchak & Stone, 2008). In this study, the authors had clinicians use their common ultrasound treatment parameters in the clinical setting compared to the recommended treatment parameters to achieve vigorous intramuscular heating. Ten subjects had a thermocouple inserted 3 cm below ½ skinfold thickness of their left calf. The observed clinical parameters were determined by a pilot study of practicing clinicians based upon the ultrasound parameters of 1.3 W/cm² for 8 minutes with an ERA of 3.9 at 1 MHz. For the recommended research parameters, the ultrasound treatment consisted of 1.5 W/cm² for 10 minutes with a 2–3 ERA. Using a 2 × 2 repeated measures ANOVA, results supported that the recommended research treatment parameters increased tissue temperature (3.9 ± 1.6°C) greater than the observed treatment parameters (2.2 ± 0.9°C). These differences may be explained by the differences in the ERA, the intensity, and duration between the two conditions. As such, the observed
clinical parameters are not beneficial for achieving appropriate heating changes and provide evidence for more educational information on ultrasound parameters for practicing clinicians.

Ultrasound in the pulsed mode may also produce thermal effects, depending upon the parameters selected. Gallo, Draper, Brody, and Fellingham (2004) compared continuous and pulsed ultrasound with the same spatial average temporal average (SATA) intensities within the gastrocnemius muscle. Subjects had thermocouples inserted 2 cm below the surface of their calf and were given two conditions: a continuous ultrasound treatment at 3 MHz, .5 W/cm² or pulsed ultrasound at 3 MHz, 1.0 W/cm², 50% duty cycle, for 10 minutes, respectively. Using Proc Mixed with variance components measured with restricted likelihood, results indicated that the temperature increased for the continuous ultrasound from baseline to a mean of 2.8°C ± 0.8°C and 2.8°C ± 0.7°C for the pulsed ultrasound. These results showed a pulsed ultrasound treatment with similar SATA can produce almost identical thermal variations in the muscle as compared to continuous ultrasound. These thermal effects provide evidence that pulsed ultrasound treatments, used for nonthermal effects, can indeed increase muscle tissue temperature, similar to continuous ultrasound. Therefore, when using pulsed ultrasound, clinicians need to scrutinize the ultrasound pulsed parameters in order to provide a treatment without thermal effects.

In summary, many factors make a significant contribution to the heating effects using ultrasound. The size of the treatment area should be 3–4 times the ERA of the ultrasound transducer for more uniformed heating in the targeted tissues. The frequency of ultrasound is related to the depth of penetration, with higher frequencies used for more
superficial tissues (less than 2.5 cm) and the lower frequencies used for tissues at greater depths (from 2.5 to 5.0 cm). The amounts of heat obtained in the tissues are dependent not only on the frequency but the intensity of the ultrasound energy, with the higher the intensity, the faster the heating of the tissue. Heating of tissues is also related to the depth, with heating rates higher for more superficial compared to greater tissue depths. The use of cold prior to ultrasound treatments impairs the heat obtained in the targeted tissues, while adding a heat pack appears to accentuate the heating effects of ultrasound.

Ultrasound and Tissue Extensibility

The increase in elastic properties of muscle tissue as a result of temperature increases has been studied with ultrasound. Draper, Anderson, Schulthies, and Ricard (1998) examined ankle dorsiflexion range of motion by heating the triceps surae muscle. Forty subjects were randomly assigned to two groups with one group stretching without ultrasound and another group stretching with ultrasound. Range of motion was measured with an inclinometer before and after treatment. The ultrasound was delivered using an Omnisound 3000C unit at 3 MHz, 1.5 W/cm² for 7 minutes. While both groups increased their range of motion, the ultrasound plus stretching group demonstrated a statistically significant increase versus the stretching alone group. Using a $2 \times 3 \times 10$ repeated measures ANOVA, results indicated that heating the muscle with ultrasound is effective in increasing elastic components of the muscle tissue based upon range of motion of the whole muscle, but the amount of extensibility of the elastic components of the muscle (muscle coverings) remains speculative.
The effects of ultrasound to elicit elasticity in ligaments were studied by Reed, Ashikaga, Fleming, and Zimny (2000). In their study, 21 subjects underwent an ultrasound treatment using 3 MHz, 1.25 W/cm$^2$ for 2.5 minutes or sham treatment to determine the heating rates, temperatures, and heating duration on the knee medial collateral ligament. Laxity of the ligament was measured two times prior (0 and 5 minutes) and three times after treatments (10, 25, and 40 minutes). Compared to the pretreatment trials (0 and 5 minutes), results showed that valgus displacements were greatest at minutes 25 and 40. With regard to ultrasound and stretching, the valgus displacements increased from 9.24° to 10.48° compared to stretching and sham ultrasound (8.95° to 10.0°). These results demonstrated that heating the ligament did not result in significant increases in valgus values and that the use of ultrasound and stretching may not be any more effective than stretching alone.

While ultrasound may increase the extensibility of muscle fibers and connective tissue, the length of time that temperature stays elevated at the desired temperatures to produce this effect must be known. The time the temperature stays at the desired thermal range after treatment is termed the “stretching window” since this is the time when the heat in the connective tissue causes increases in elasticity (Draper & Ricard, 1995). Draper and Ricard used 3 MHz ultrasound treatments to find the stretching window. Subjects underwent an ultrasound treatment at 3 MHz for a 5°C temperature change, recording both time and temperature from baseline. Using a nonlinear stepwise regression to predict temperature decay as a function of time, results indicated that on average it takes about 3.3 minutes following an ultrasound treatment to return to baseline measures. In a follow-up study, 21 subjects underwent an ultrasound treatment using 1 MHz at 1.5
W/cm² with an Omnisound 3000 until the temperature achieved a 4°C increase from baseline (Rose et al., 1996). Thermistors were inserted at 2.5 and 5 cm deep into the triceps surae muscle and temperature was recorded every 30 seconds. Once the critical temperature of 4°C was achieved, the temperature decay was recorded until baseline temperatures were obtained. Using a stepwise nonlinear multiple regression, results of their study showed that once the 4°C was reached, it took approximately 21.4 ± 4.8 minutes to return to baseline for the 2.5 cm depth and 21.2 ± 4.6 minutes for the 5 cm depth. At the 2.5 cm depth, the times for decay expressed as a drop per each degree were 1°C = 2:34, 2°C = 6:35, 3°C = 12:10, and 4°C = 21:14. For the 5 cm depth, the changes were 1°C = 2:31, 2°C = 6:50, 3°C = 14:32, and 4°C = 27:49. While there were no significant differences between depths, the thermal decay for 1 MHz was slower than 3 MHz (Draper & Ricard, 1995). Based upon the results, it was recommended that in order to capitalize on the thermal effects to increase tissue extensibility, stretching should be initiated within the last few minutes while applying the ultrasound treatment instead of waiting until the completion of treatment since the temperature after treatment begins to drop quickly.

In summary, it appears that ultrasound energy affects tissues higher in protein content, like tendons, resulting in the ability to increase temperature effectively and quicker than muscle tissue, but does not affect ligamentous structures as effectively. The length of time that the tissues remain elevated allows tissue to retain heat and increase the extensibility of the fibers, resulting in better elongation of the tissues. It was also recommended that stretching of tissues occur during ultrasound treatment rather than after
Subcutaneous Tissue and Thermal Effects

As discussed previously, energy is transmitted into the tissues and creates movement of molecules which, in turn, causes friction and temperature increases. According to Draper and Sunderland (1993), ultrasound energy should penetrate through tissues high in water content and be absorbed in high protein content tissues. As such, ultrasound energy should readily penetrate through subcutaneous adipose tissue to reach underlying tissue structures, such as muscle, tendon, nerve, and bone where the energy is absorbed (Dyson, 1987).

The amount of subcutaneous adipose or fat thickness, however, may make a significant impact as to the amount of energy transmitted or absorbed by the underlying tissues. In a study by Draper and Sunderland (1993), 20 subjects received a treatment with ultrasound at 1.0 MHz, at 1.5 W/cm$^2$ for 10 minutes or until no temperature changes occurred after three consecutive readings (measured every 30 seconds). Subcutaneous skinfold (adipose) thickness was measured using skin calipers on the right calf and divided by 2 to represent the thickness of fat under the skin surface. A temperature probe was inserted at 3 cm depth on all subjects. Subjects were split into two separate groups, those with more or and those with less than 10 mm skinfold thickness. Subjects in the less than 10 mm thickness group had an average temperature increase of $4.9 \pm 1.0^\circ$C compared to $4.8 \pm 1.7^\circ$C for the greater than 10 mm group, which was nonsignificant. Due to the large skinfold variability, a regression analysis was conducted to examine the
relationship of subcutaneous adipose and tissue temperature increases. A small
nonsignificant but positive correlation \( r = .128 \) was found. The results demonstrated
that the subcutaneous adipose thickness does not serve as a barrier to raise temperature of
muscle tissue at a depth of 3 cm. However, this study utilized 1 MHz frequency, which is
purported to change temperature of deeper tissues versus superficial tissue. In addition,
the time it took to raise the temperature was not recorded.

Later, Castel et al. (1998) examined the temperature changes in subcutaneous
adipose tissue. In their study, 10 subjects underwent ultrasound treatments with settings at
3 MHz, 1 W/cm\(^2\), for 10 minutes with an Omnisound 3000. Temperature was taken
within the adipose tissue over the muscle belly of the left triceps muscle, but the specific
depth was not provided. The temperature at the end of the treatment reached a mean peak
of 41.4°C, with an average increase from baseline to peak about 8.4°C. After treatment,
the temperature decreased 4.8°C at 5 minutes post and 6.4°C at 10 minutes post
treatment. These results suggested that adipose can reach temperatures similar to or
greater than muscle with 3 MHz ultrasound treatments, even though the structural
components of these tissues are dissimilar, speculating that subcutaneous adipose may
absorb more energy than previously thought.

While only two studies specifically focused on ultrasound heating and
subcutaneous thickness properties, another study examined the effects of skin temperature
and adipose thickness during cryotherapy use (Jutte, Merrick, Ingersoll, & Edwards,
2001). Fifteen subjects underwent a 30-minute ice bag treatment followed by a re-
warming phase after removal of the ice bag for 120 minutes. A multiple linear regression
with five independent variables (body core temperature, skin temperature, subcutaneous
adipose thickness, room temperature, and time) was used to predict the intramuscular temperature of the anterior thigh based upon skin temperature. An intramuscular thermocouple was inserted to a depth of 2 cm, while surface thermocouples were placed within the treatment area of the thigh. Using regression analysis, results indicated that the intramuscular temperatures decreased over 8°C, while the skin temperature decreased approximately 27°C over the 30-minute treatment period. While no clear single predictor explained the intramuscular temperature, the combined predictors explained about 76% of the intramuscular temperature changes. Adipose thickness accounted for 14% of the intramuscular changes, making it a weak predictor. However, adipose thickness, used in this cryotherapy study, was acting as an insulator to the tissue cooling effects instead of transmission of heat energy.

In a similar study examining adipose thickness and cryotherapy, 47 subjects were divided into four groups based upon adipose thickness (0–10, 11–20, 21–30, and 31–40 mm thickness) to further examine the range of thickness on intramuscular temperature changes (Otte et al., 2002). An ice bag was placed on the anterior thigh with an intramuscular probe inserted at 1 cm below ½ skinfold thickness. The ice bag was applied until 7°C reduction from baseline temperature was achieved. The time to achieve the 7°C change differed across the groups, with the thicker groups requiring more time to cool the intramuscular tissue. Using an ANOVA, results indicated that the thicker the subcutaneous tissue, the more time required to reach the temperature, necessitating the alterations of treatment time based upon body composition of the patient. Similarly, Lowdon and Moore (1975) studied the effects of cold on adipose thickness and intramuscular temperatures and found that the temperature changes in the muscle tissue
was inversely related to skinfold ($r = .69$) and circumference of the extremity ($r = .80$) based upon mean temperature changes using a regression model. Johnson et al. (1979) also showed similar relationships between skin and intramuscular temperatures. These results also suggested that the subcutaneous thickness alters the thermal energy (cold) and the effects of the intramuscular tissue changes.

A final study investigated the relationship between the amount of adipose and intramuscular temperature during and after a 20-minute ice bag application (Myrer, Myrer, Measom, Fellingham, & Evers, 2001). The subjects in the study were divided into three equal groups based upon adipose thickness—8 mm or less, 10–18 mm, and greater than 20 mm. The intramuscular temperature was measured at two depths, 1 and 3 cm below the adipose layer. Using an MANOVA, results indicated that there were significantly different intramuscular temperatures in all three adipose groups at the 1 cm and 3 cm depths. The rate to which the intramuscular temperature tissues cooled was vastly different depending upon adipose thickness. The thinner the adipose layer, the faster it was cooled. Results from the study, although pertaining to cold modality treatments, suggested that the adipose thickness played a significant factor in the temperature and rate of thermal conductivity. The authors provided guidelines for duration of treatments and recommended for cold treatments that with 20 mm or less of subcutaneous tissue, the time for ice application should be 25 minutes; for 20–30 mm of subcutaneous tissue, the time for ice application should be 40 minutes; and 30–40 mm of subcutaneous tissue should have treatment times of approximately 60 minutes.

In summary, adipose is purported to have little, if any, effect on ultrasound treatments. However, examination of previous studies revealed controversies as to the
extent adipose has on absorption or transmission of ultrasound energy. It appears that subcutaneous adipose can heat similarly to muscle tissue, but when heating tissues at greater depths with lower frequencies, adipose does not hinder energy absorption. Cold therapy treatments, conversely, have shown that subcutaneous adipose has a significant insulating effect, with the greater the adipose thickness, the more time needed to cool the underlying tissues.

Skin Temperature and Thermal Effects

For many years, skin temperature measurements have been used in modality research. While the literature demonstrated that changes in skin temperature occurred during various treatments, the use of skin temperature to predict intramuscular temperature may be based upon the type of heating or cooling medium utilized for the treatment. A study conducted by Merrick, Knight, Ingersoll, and Potteiger (1993) postulated that skin and intramuscular temperatures may not be as highly correlated as previously thought. In Merrick et al. (1993) and a study by Johnson et al. (1979), muscle and skin temperature were measured during cold applications. The length and time that the skin remained cool and how this coolness affected muscle temperature varied with the type of cold modality utilized.

In another study, skin temperature was found to be a weak predictor of intramuscular temperature changes, explaining about 21% of the variability. It was suggested, based upon the immediate effects of skin re-warming after cessation of treatment though intramuscular temperature was still decreasing, that skin temperature was not a strong predictor of intramuscular temperature (Jutte et al., 2001).
The aforementioned studies have one commonality; they measured skin temperature and made predictions of skin temperature with intramuscular temperatures using cold modalities. While skin temperature does decrease dramatically and gradually re-warms after cessation of cold treatments, the adipose layer most likely was a considerable factor in intramuscular temperatures during treatments based upon the previous reviewed studies. Ultrasound energy may pass through the adipose layers with minimal attenuation of energy, and adipose functions differently in cold applications, as opposed to acting like an insulator to modality treatments.

In summary, skin temperature has been used to predict muscle temperature, but the effectiveness varied according to the type of cold modality used and length of treatment. The adipose layer between the skin and muscle tissue also was purported to contribute to difficulties in correlations between muscle and fat. However, the research on skin temperature and muscle temperature has been conducted with cold modalities in which the adipose layer serves as an insulating barrier versus transmission of energy. It remains to be determined if skin temperature can serve as a predictor in ultrasound applications.

Ultrasound Units

There are multiple ultrasound units such as Mettler Electronics, Dynatronics, Chattanooga, and Accelerated Care Plus (Omnisound) that are used by health care professionals for treatment and rehabilitation of patients. Does selecting one manufacturer over another make a significant difference in the amount of energy transmitted to affect heating of tissues? In a study by Holcomb and Joyce (2003), two commonly selected
ultrasound units (Chattanooga Forte 400 Combo and the Accelerated Care Plus Omnisound 3000) were compared to determine if similar heating characteristics were found. Ten subjects had a thermistor microprobe inserted in the left triceps surae at 1.2 cm depth. Subjects were counterbalanced for treatment order both with identical 1.5 W/cm$^2$ at 3 MHz for 10 minutes with a 2 ERA treatment areas. Using a 2 within by 2 between mixed design ANOVA, there was a significant main effect of unit, with the Omnisound 3000 producing a greater temperature elevation approximately 2°C more than the Forte 400 Combo and at a mean rate of .58°C per minute. The authors noted that temperature changes may be a result of the BNR of the units; however, the Forte 400 Combo had a lower BNR (2.3:1) compared to the Omnisound 3000 (3.7:1). In addition, the ERA for the Forte 400 Combo was 4.6 cm$^2$ compared to the 4.9 cm$^2$ for the Omnisound 3000, both with a 5 cm$^2$ sound head. With a larger surface area found in the Omnisound 3000 transducer head (about 7%), the temperature differences calculated per percentage of transducer size did not support the tissue temperature fluctuation between the transducers. The authors suggested that further research should examine the peak area of the maximum BNR for possible causes of the temperature tissue differences.

Similarly, three different ultrasound devices, Omnisound 3000, Dynatron 950, and Excel Ultra, were compared for tissue heating effects (Merrick, Bernard, et al., 2003). Six subjects were given identical ultrasound treatments of 3 MHz, at 1.5 W/cm$^2$ for 10 minutes in a 2 ERA treatment site in a counterbalance design over several days. Intramuscular temperatures were measured at depth of 1.6 cm from the surface of the left calf at two distinct time points: at 6 minutes of treatment and at the end of the total 10-minute treatment time. Results using a MANOVA showed that the Omnisound treatments
were discontinued at approximately 6 minutes due to subject discomfort, which equated to approximately 41°C intramuscular temperature, while the other two units also failed to reach a 40°C threshold average for all subjects. These results show that the Omnisound 3000 had significantly greater temperatures at the 6-minute treatment time than the other two units, with both the Dynatron and Excel units not differing from each other. The authors suggested that since the heating parameters used by clinicians are based upon studies conducted using the Omnisound 3000, following these temperature heating guidelines and rates may not be as accurate with other units in raising tissue temperature to a critical temperature set point.

In summary, it appears that ultrasound heating effects may be dependent upon the type of manufacturer selected. Predominantly, all ultrasound research utilizes the Omnisound unit and heating temperatures and heating rates were conducted with an Omnisound unit. Therefore, ultrasound treatments should be given with the Omnisound unit if clinicians want to follow the recommended temperature guidelines. However, ultrasound units made by other manufacturers can still be applied, but adjustments may have to be made in the time and heating temperatures. More research is needed to compare manufacturers.

Ultrasound and Coupling Mediums

In order for adequate transfer of energy from the transducer to the underlying tissues, a coupling medium must be present that allows for the transfer of the energy from the transducer to the tissues. Clinicians most commonly use several types of coupling mediums, such as gels, oils, or lotions, with the most common medium being ultrasonic
gels. Ultrasonic gels are 99% water, which allows the ultrasound energy to pass through the gel to the tissues easily. If there is no coupling medium between the transducer and the skin, the ultrasound energy will not penetrate easily.

For irregular body surfaces, such as ankles, wrists, or other bony surfaces, direct transducer application is difficult since there is ununiformed transducer head contact. To help provide more contact surface over these area, gel pads are often used. The transducer is applied to the gel pad that sits directly over the irregular shaped body part. This direct contact theoretically allows the transducer head to remain in total contact with the gel pad, which in turn delivers the energy to the tissues underneath. A study by Merrick, Mihalyov, Roethemeier, Cordova, and Ingersoll (2002) compared the intramuscular tissue temperature changes during ultrasound application with gel pads or standard direct contact. Thirteen subjects received an ultrasound treatment using a Forte 200 unit set at 1 MHz, 1.5 W/cm² for 7 minutes over a treatment area two times the size of the transducer head. Intramuscular temperatures were measured by a thermocouple inserted into the right calf at a depth of 3 cm below the skin surface. Subjects then received either a standard ultrasound treatment with ultrasonic gel or a treatment with a gel pad in a counterbalanced design. Results using an ANCOVA (with baseline temperature as the covariate) indicated that peak temperatures between the two treatments did not differ significantly, with normal gel reaching 39.2 ± 2.4°C, while the gel pad had a peak temperature of 39.4 ± 1.5°C. These results indicated that either coupling medium can elevate intramuscular temperatures consistently. The overall temperatures during the 7-minute study raised intramuscular temperature only approximately 2°C above baseline
and longer treatments or higher temperatures that are normally administered with ultrasound are in question.

Another coupling medium known as T-prep, which contains 1% methyl nicotinate (MN) and reportedly helps hydrate the outer surface of the skin and allows better passage of ultrasound energy, was studied by Gulick, Ingram, Krammes, and Wilds (2005). In this study, T-Prep was compared with regular aquasonic gel temperature changes during a 3 MHz ultrasound treatment. Thirty subjects were given treatments of either T-Prep or aquasonic gel at 1.0 W/cm² for 15 minutes. Temperature was measured at a depth of 2.5 cm of the calf. Results using repeated measures ANOVA indicated no differences in temperatures for either type of gel. T-Prep gel reached an overall 1.8°C increase at 15 minutes compared to 1.5°C for aquasonic gel. These results revealed that the gel type was not a significant factor in the overall tissue temperature increase and each gel was determined to be adequate in its abilities to effectively deliver ultrasonic energy.

Coupling mediums used for ultrasound therapy have also included topical analgesics. Topical analgesics are often used to provide a warm sensation to the treatment area in order to relax tissue contractile properties and provide pain relief via nerve inhibition. Analgesics also come in various formulations, similar to pharmaceutical agents, but the transmission properties with topical analgesics is limited. A comparison of ultrasound gel and two commonly used analgesic agents (Biofreeze and Nature’s Chemist) were compared to determine intramuscular temperatures to determine which coupling medium produces the desired critical temperature (Myrer, Measom, & Fellingham, 2001). Forty subjects were randomly assigned to one of four conditions: ultrasound gel, Nature’s Chemist, Biofreeze, and sham. The subject’s left calf was
cleaned and shaved and a thermocouple was inserted at a depth of 1 cm plus ½ skinfold thickness. Subjects were given a treatment with set parameters of 1.0 W/cm² at 3 MHz for 10 minutes. Results indicated that the experimental groups had higher temperatures compared to the sham group but did not differ from each other. In terms of effectiveness, all gel groups had similar temperature increases in the muscle, which demonstrated equal effectiveness for raising muscle temperature.

Another coupling compound, called Flex-All, is commonly used for alleviation of pain or muscle aches. Flex-All is composed of about 7% menthol and classified as a topical analgesic. Flex-All is commonly applied to the area via massage, but has been claimed to be an effective coupling medium for ultrasound treatments. In a study by Ashton, Draper, and Myrer (1998), three ultrasound treatments were given to determine which coupling medium (50% Flex-All with 50% gel, 100% gel, and sham with 100% Flex-All) would be more effective in raising muscle temperature. Fifteen subjects had microprobes inserted at 3 and 5 cm in their left calf and received one of the three treatments with an Omnisound 3000 at 1MHz, 1.5W/cm² for 10 minutes. At the 3 cm depth, the gel coupling medium increased 3.2°C compared to the 50% mixture (2.6°C). At the 5 cm depth, the gel increased 2.17°C compared to the Flex-All mixture (1.80°C). Results using repeated measures ANOVA showed that the 100% gel had higher muscle temperatures than the 50% mixture. However, when subjects reported heating sensation, the 50% mixture was rated warmer than the 100% gel comparison.

The temperature of the ultrasound gel may also be an important consideration for clinical treatments. In a study by Oshikoya et al. (2000), ultrasound gels were applied at different temperatures to determine if these coupling mediums would affect intramuscular
temperatures. Three gel temperatures (cold, 18°C; room, 25°C; and heated, 39°C) were applied to 18 subjects over three time periods, and muscle temperature was recorded at a depth of 5 cm below the surface. An Omnisound 3000 was set at 1 MHz at 1.5 W/cm² until the muscle temperature reached 4°C above baseline. Results using repeated measures ANOVA indicated that the mean temperature increase across all subjects was 35.47 ±.74°C. Final temperature readings indicated that the rate at which the temperature reached the critical 4°C change was faster for the room gel than the other two gel conditions. Based upon the results, it appears that the room temperature allowed the rate and temperature to rise quicker than the other two conditions. In addition, there is no apparent rationale for clinicians to use either cold or heated gel with ultrasound treatments for clinical outcomes, especially when treatment time and patient comfort is taken into account.

In summary, ultrasound treatments must utilize a coupling medium, usually water soluble gels, to transfer the ultrasound energy to the tissues. Other types of gels can be used but may affect the delivery of ultrasound energy, with thicker gels resulting in less transmission of ultrasound energy. The use of room temperature gels also has been reported to allow the most energy into the tissues.

Ultrasound Usage

Ultrasound is a commonly used modality in the allied health clinical setting for a variety of medical conditions on a wide range of patients. A study by Warden and McMeeken (2002) investigated the usage, dosage, and training of ultrasound treatments with physiotherapists. Results showed that only 1% of all surveyed physiotherapists did
not use ultrasound in their clinical practice, while 84% and 12% used it daily and weekly, respectively. Of those who did use ultrasound, 88% used it for chronic injuries and 86% for acute injuries, with the knee and ankle regions used predominantly. While the use of ultrasound remains high, the rationale for ultrasound settings remains speculative. For acute conditions, physiotherapists used intensity (dosage) between .51 and 1.5 W/cm² and 1.01 to 2.0 W/cm² for chronic conditions. When asked how the dosage was selected, the pathology being treated (87%), the type of tissue treated (80%), the depth of tissue treated (75%), and the region being treated (72%) were deemed as the most important factors. Of interest, only 35% of physiotherapists surveyed used research evidence for selecting their dosages as compared to 83% who relied on their undergraduate training experience.

In a similar study, Dutch physical therapists were asked to respond to a survey over a 3-year period about their use of ultrasound on their patients (Roebroeck, Dekker, & Oostendorp, 1998). The physical therapists described that the most common reasons to use ultrasound were for shoulder, knee, and ankle injuries. The use of ultrasound seems to occur most often during the first 3 weeks (35%) of treatment compared to long-term treatments of over 6 weeks (27%) in length. Patients who received ultrasound also had lower frequency of manual therapy techniques (3.7% occurrence) compared to non-ultrasound treatment groups (13.5% occurrence).

Physical therapists that specialized in orthopedics were also surveyed about their perceived clinical importance of ultrasound in their clinical practice (Wong et al., 2007). Their 77-question survey specifically addressed usage, importance, and parameters used to manage physical impairments with ultrasound based upon physical therapists with Orthopaedic Certified Specialists (OCS) designation from a list provided by the American
Physical Therapy Association (APTA). Results indicated that OCS physical therapists use ultrasound for pain (49.3%), soft tissue inflammation (83.6%), tissue extensibility (70.9%) and scar tissue remodeling (68.8%). The respondents stated that continuous ultrasound was used most frequently with increasing tissue extensibility (93.6%) and pain control (75%), while a pulsed method was used for decreasing soft tissue swelling (82.3%). Intensity settings most frequently used range between .1 to 3.3 W/cm² for superficial tissues and .4 to 4.0 W/cm² for deep tissues.

In summary, ultrasound treatments have been used frequently with the treatment of injuries in the sports medicine profession. The parameters used with ultrasound treatments varied according to the type of injury and the injury outcomes. In most cases, ultrasound was used with painful conditions and to help with the extensibility of tissues. In addition, most clinicians use ultrasound in the continuous mode to increase tissue temperature versus the pulsed mode, which often is used for nonthermal effects.

Statistical Analysis Techniques

The aforementioned studies on ultrasound research utilized several different types of analysis. An overwhelming amount of research used ANOVA or repeated measures ANOVA analysis techniques with four studies using regression analysis (Draper & Ricard, 1995; Jutte et al., 2001; Lowdon & Moore, 1975; Rose et al., 1996), one using MMRM (Gallo et al., 2004), and no known studies using HLM. In ultrasound research, the temperature collected represents repeated measures. This means that the subjects will be exposed to each treatment interval, which minimizes any confounding outcomes based upon uncontrolled variables, such as environment. Therefore, the error of the sum of
squares with the treatment should be lower than subjects randomly assigned to groups, which results in more statistical power. A main advantage of a repeated measures design is that all subjects experience the same treatment and any observed differences of the treatment should be based upon the effects of this treatment. However, while trying to control variability, repeated measures models do not always control for the individual differences to the treatment variable (Wells, 1998).

In the review of the ultrasound literature, an overwhelming majority of the analysis was conducted using ANOVA or regression techniques; however, the data appear to be analyzed using a mean ending or specific time point value rather than trying to partition the repeated nature of the data or the variability of the subject over time. As such, several different analysis techniques were employed in this dissertation to examine the relationship of the independent variables (skin temperature and skinfold thickness) during the ultrasound treatment as specific intramuscular depths. The analytical models utilized are OLS regression, hierarchical linear models (HLM), and mixed models repeated measures (MMRM). OLS regression was selected since the model historically has been used to predict an outcome of the independent variable upon the dependent variable of interest and is the common type of model used in ultrasound research when trying to predict outcomes. If the goal is to determine an ending or specific temperature time point in ultrasound research, then the use of regression is appropriate. However, if the goal is to determine growth rates and predictions of these growth rates with other variables, then the use of HLM or MMRM may be more appropriate since regression cannot conduct growth curves. Therefore, HLM and MMRM were selected as two other comparison models because these models account for within- and between-subject effects.
and produce growth rates. The use of HLM and MMRM were also selected because they have not been found in the ultrasound literature, perhaps due to unfamiliarity of these techniques.

To begin, regression techniques are useful not only for controlling any confounding variable, but also to predict the “power” of sets of predictor variables or single variable with the dependent variable (Hoyt et al., 2008). Regression provides information about not only the overall model used in the analysis, but also the contribution of the variables within the overall model. Regression uses values of two variables, $X$ and $Y$ to construct a model to predict a given dependent value, $Y$, based upon the contributing factor of the $X$ variable, commonly in a linear equation of $Y = \beta_0 + \beta_1 x_1 + \epsilon$, where $Y$ is the response variable, $\beta_0$ is the intercept, $\beta_1$ = coefficient, $x_1$ = predictor variable contribution, and $\epsilon$ = error term. There are several assumptions for OLS regression. The $X$ and $Y$ variables should be measures at the interval or rational level, and their relationship to each other should be linear, suggesting that the variances across different time are equal and covariances are equal (compound symmetry) (Wu, 1996). Regression analysis uses ANOVA modeling techniques, with the significance of the $F$-tests and computational methods, based upon a constant correlation among the multiple measures of the repeated variable. OLS regression minimizes the sum of square residuals and is unbiased if the errors have a finite variance and are uncorrelated with the regressors. For linear regression, the coefficient estimates closely resemble the correlation coefficient. If there is more than one independent variable, and the independent variables are highly correlated together, called multicollinearity (usually when bivariate correlation $\geq 0.9$), the regression coefficients may not show significance because of the large
standard errors. Outliers in the data also tend to skew the results in regression. Outliers can be examined with specific tests or by viewing the residual scatter plots for normality, linearity, and homoscedasticity. Normality refers to the normally distributed residuals about the predicted dependent variable scores, linearity shows residuals with a straight-line relationship with the predicted dependent variables, and homoscedasticity represents the variance of the residuals. When the residuals violate these assumptions, the regression technique may not be appropriate for the analysis.

HLM, on the other hand, is designed to examine data and trends associated with the individual in the level 1 model, then examine how these trends are affected by the individual characteristics in the level 2 model (Wu, 1996). HLM uses the repeated measures units (in this study, the units of time are nested within individuals) to examine patterns of change. HLM stipulates a hierarchical structure, meaning that multiple time point measurements are “nested” within individuals and each individual is allowed to have his or her own growth curve, which can vary across individuals (van der Leeden, Vrijburg, & de Leeuw, 1996). In other words, a growth trajectory is determined, and then this model is used to determine the between-individual factors to measure not only the rate but the pattern of change over time (Bryk & Raudenbush, 1987). HLM examines the changes in the trends of the individuals, then examines the variations between individuals in the parameter estimates that may show patterns of changes since these individuals’ changes differ across the subjects (Wu, 1996). HLM, therefore, can examine each subject’s trends, the overall group’s trend or trajectory, and the variance of the individual around the mean or average of the trend, and can predict the individual’s differences in the trends (Hernandez-Lloreda et al., 2003). Of equal importance, HLM can be used when
the data collection points are unequal. In ultrasound research, time points are seldom equal, with some subjects taking longer to increase temperature compared to others. The estimation methods of HLM therefore can examine the effects within the subject, model these effects across the subject, and also partition variance components (Wu, 1996).

The MMRM model is very similar to the HLM model. MMRM produces a covariance structure when there are missing data by a generalized least squares computational method compared to OLS. Another advantage of using MMRM is the ability to examine the “fit” of the growth curves, which means comparisons can be made with the rate of the growth (slope). Growth can be defined as a measurement of one variable for the same person over several consecutive points in time (van der Leeden et al., 1996). Examination of growth curves allows the researcher to explore differences in initial values and rate of growth instead of comparing means across time points and experimental treatments (Ugrinowitsch, Fellingham, & Ricard, 2004). The major difference in analysis techniques for this dissertation is that the MMRM was based upon dividing the treatment into 10 equal time points, while HLM did not impose these restrictions.

In summary, OLS regression, HLM, and MMRM use similar computation to estimate repeated measures data. Regression tries to “predict” the outcome and is based upon mean scores. It provides estimates of group differences and can test for group trends. However, OLS regression cannot provide information that could affect individual rate of changes (O’Connell & McCoach, 2004). Finally, if violations of normality are found, the results may be biased. Both HLM and MMRM examine individual and group trends over time and measure the growth or slope of the data to help explore and interpret
how the data can vary both within and between persons. Both HLM and MMRM are effective in estimating how an individual’s growth occurs over the treatment period and allows estimations for the coefficient to be adjusted for the individual effects versus a mean group effect utilized in OLS regression.
CHAPTER III

RESEARCH DESIGN

There are several purposes of this study: (1) to determine the overall absolute and relative intramuscular and skin heating temperatures and heating rates, (2) to determine if skin temperature can be used to predict intramuscular temperature, and (3) to determine if skinfold thickness affects the heating of intramuscular tissues using therapeutic 3 MHz ultrasound. To make appropriate decisions for the research question outcomes, comparisons of the results for all three analytical methods (OLS regression, HLM, and MMRM) will be conducted but not discussed in this chapter.

This chapter is divided into sections. The first section explains the pilot study to determine the methodology and sample size calculation. The subsequent sections explain the exact procedures and statistical analysis methods.

Pilot Study

To begin, a pilot study was conducted over two sessions to determine efficacy and feasibility of the procedures for conducting this study, specifically examining surface and intramuscular temperatures, and to determine sample size. Parameters for the pilot study were based upon using a 3 MHz ultrasound treatment for a 2°C change. An Omnisound ultrasound unit was utilized to deliver the treatment using the preset time of 3:36, which was calculated from the heating rates of 3 MHz, at 1.0 W/cm² at the intramuscular depth
of 1.5 mm into the calf muscle as specified by Draper, Castel, and Castel (1995). Skin temperature was also recorded for these sessions, since skin temperature is used as a predictor in determining heating of the intramuscular tissues. Two different subjects were used for the pilot study with different skinfold measures. Subject one had a skinfold of 20 mm and subject two had a skinfold of 12 mm. Both subjects were treated with the identical ultrasound settings, but only subject two had an intramuscular probe inserted.

For subject one, skin temperature was measured over two separate occasions with a washout period for skin temperatures to return to baseline. For the first session, baseline skin temperature was 26.5°C and reached a temperature of 33.0°C at the end of the treatment time of 3:36 based upon the preset time of the ultrasound unit for a 2°C temperature change (there is no 3°C temperature change setting). For the second session, baseline skin temperature was 25.4°C and reached a temperature of 31.5°C at the end of the 3:36 treatment time.

For subject two, both skin and intramuscular temperatures were recorded during ultrasound treatment. Treatment was set using the same ultrasound parameters but without a time limit to reach the 2°C. The skin temperature increased from 26.2°C to 37.7°C, while intramuscular temperature at 3 cm of depth went from 36.6°C to 38.6°C after a treatment time of 5 minutes to raise the intramuscular temperature a total of 2°C.

To determine if just moving the ultrasound head over the skin surface alters tissue temperature of the skin by friction alone, the preset ultrasound treatment parameters with a time of 3:36 was performed on a subject without turning on the ultrasound machine. Temperature at the starting point was 26.6°C and ending point was 26.5°C, showing that
the friction of the ultrasound head over the skin does not increase or alter skin surface
temperature during the ultrasound treatment.

These results help support the need to further examine the relationship of skin
temperature and intramuscular heating alterations with ultrasound treatments. In addition,
the depth and overall heating of the muscles seem to vary, suggesting the influence of
other factors, namely depth and tissue characteristics.

Power Analysis

An *a priori* power analysis was calculated based upon the pilot results and
previous studies (Gallo et al., 2004; Rose et al., 1996; Weaver et al., 2006) for
determining the sample size. In Weaver et al. (2006), the baseline intramuscular
temperature was 37.8°C ± 0.8°C and after treatment was 42.9 ± 1.9°C using 1 MHz
ultrasound. In the study conducted by Rose et al. (1996), the baseline temperature was
34.7°C ± 1.1°C and after treatment using 1 MHz, the temperature increased to 38.7°C ±
1.6°C. Finally, in Gallo et al. (2004), the temperature at baseline 35.5°C ± 0.9°C was
increased to 38.4 ± 0.8 °C after treatment with 3 MHz.

A free downloaded sample size calculator, GPower 3.0.10 (University Kiel,
Germany), was used to estimate sample size based upon a generalized linear model since
temperature appears to rise in a linear fashion. A multiple regression omnibus ($R^2$
deviation from zero) prediction model with two predictors to determine intramuscular
temperature changes was selected as the appropriate model. The first predictor was skin
temperature and the second predictor was the skinfold thickness. The alpha was set at .05
and 1-β (power) was set at .80. Results of the power analysis, with a critical $F$ calculated
at 3.13, produced a needed subject sample size of 31 for the study (see Appendix A) under the assumption of a linear model. Therefore, a total of 33 subjects were recruited to complete the study, two more than the model suggestion, to account for subject attrition.

Subject Selection

For the purposes of this study, the population selected consisted of students, faculty, or staff at Western Michigan University. This population was chosen due to convenience. Although a convenience sample, the population does represent normal human physiology inherent in the population as a whole.

All subjects met the following conditions in order to be selected for participation; they were within the ages of 18–45 years old, they were free from any current trauma to the lower leg (calf region) or recent leg injuries (within the past 6 months), and they had no known infection of the lower leg and no vascular or nervous conditions. In addition, subjects could not have any reliance on a pacemaker or be pregnant (female subjects only). A research flyer containing the study’s purpose and inclusionary and exclusionary criteria was posted in the Student Recreation Center to recruit subjects (Appendix B). Subjects who met the aforementioned criteria were instructed to contact the primary researcher for any further explanations. Subjects who were interested in participating were scheduled for a 90-minute appointment during time that was convenient for both the researcher and subject. Subjects were also informed to shave their calf prior to data collection.
Procedures

Subject Preparation

Before data collection, subjects read and signed an approved Western Michigan University Human Subjects Institutional Review Board informed consent (Appendices C and D), followed by completing a health history questionnaire (Appendix E). Demographic information (i.e., height, weight, skinfold thickness) was collected after completion of the aforementioned forms. Subjects then were placed on a treatment table in the prone position with the ankles over the back edge of the table. The left calf was shaved (if not done prior) and cleaned with 70% isopropyl gauze pad. Calf skinfold and calf circumference were measured. Calf skinfold was measured using skinfold calipers (Lange, Inc, Santa Cruz, CA) at the mid-section (widest) of the calf. Skinfold measures were taken two to four times to ensure consistent readings. Interclass correlation coefficients were conducted on the consistency of the values to establish intrarater reliability. The value that occurred most often was the value recorded for the subject. Results show that the ICC was .981.

To help ensure reliability, another trained athletic trainer in skinfold measurement independently measured three subjects, three times each. Comparisons between the researcher and the certified athletic trainer resulted in an interrater agreement rate of 66% for the actual values for three measurements over two subjects (Table 1). However, when examining the measurements, the values were off by only 1 mm on three separate occasions, suggesting that the precision was within acceptable parameters, especially for
the complexity of measurement. This consistency of measures ensured that the depth for the relative site was calculated based upon accurate subcutaneous skinfold thickness.

Table 1

*Interrater Skinfold Measurement Accuracy*

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*Note.* All trials measured in mm.

Calf circumference for each subject was measured using a flexible tape measure that was wrapped around the calf at the mid-section or widest part. Measurements were made to the nearest millimeter.

*Thermocouple Placement Sites*

A depth template (Figure 1) was used to measure the absolute depth, which was defined as 1.5 cm from the surface of the treatment area.

The depth was measured by placing the template over the mid portion of the left calf and measuring down the medial side of the calf to 1.5 cm. A mark, using a permanent pen, was placed on the medial portion of the mid-calf at the corresponding depth. Approximately 5 mm caudally to the absolute depth, the relative depth was located. Relative depth was calculated by adding \( \frac{1}{2} \) the skinfold thickness of the subject to the absolute depth (1.5 cm). For example, for a skinfold thickness of 10 mm, the relative
depth would be determined by adding ½ the skinfold thickness (5 mm) to the absolute depth (1.5 cm) for a relative depth of 2.0 cm.

Figure 1. Depth Template

After measuring the absolute and relative depths, a treatment template was traced on the superior or top of the calf for the designated treatment site. The template was fabricated based upon two times the effective radiating area (ERA) or approximately 7.2 cm × 3.5 cm based upon a 5 cm² ultrasound head size (Figure 2). The template was centered on the calf based upon the location of the absolute and relative depth markings.

Thermocouple Insertion

Two temperature probes (Physitemp MT-26-gauge, 4 cm thermocouples) that were soaked in Cidex® for a minimum of 24 hours were used to measure absolute and relative depth calf temperatures. Before thermocouple insertion, each subject’s skin was cleansed again using 70% isopropyl alcohol pads over the depth marking sites. For the
Figure 2. Surface Treatment Template

absolute thermocouple insertion, cold spray (Cramer, Inc., Gardner, KS) was sprayed over the insertion site for approximately 10–15 seconds or until the area became white in appearance in order to numb (but not freeze) the superficial tissues to minimize discomfort. The absolute depth probe was then inserted at the absolute mark until the end of the 4 cm probe reached the border of the skin. Gebauer’s Spray and Stretch cold spray (Gebauer Company, Cleveland, OH) was used to numb the relative depth site in the same manner as the absolute depth site. Gebauer spray was used for the relative depth because of the stream and pinpoint accuracy of the cold spray. Gebauer spray was administered at the relative depth mark for 10–15 seconds or until the area became white in appearance in order to numb (but not freeze) the superficial tissues to minimize discomfort. The relative depth thermocouple was then inserted.

After the insertion of the two thermocouples, a surface temperature wire was affixed to the treatment area approximately 1 cm from one edge of the template ERA. The wire was affixed to the skin using Tegaderm (3M, St. Paul, MN) transparent film
dressing that was cut into a small 2 × 2 cm square. The superficial temperature wire and Tegaderm dressing did not cover the surface above the two thermocouples. The surface wire and thermocouples were attached to the Thermes USB (Physitemp Instruments, Inc., Clifton, NJ) temperature unit. Upon insertion of the two thermocouples and affixed surface temperature wire, 5 mL of Aquasonic coupling gel (Parker Laboratories, Inc., Fairfield, NJ) was applied within the treatment ERA template (Figure 3). The Aquasonic coupling gel temperature used throughout the study ranged from 21.8 to 22.3°C with an average temperature of 22.1°C.

![Figure 3. Final Preparation of Subject](image)

**Treatment Application**

After insertion of the thermocouples and surface temperature wire, baseline temperatures were recorded for 10 minutes. Temperature for all thermocouples and surface temperature wire were recorded in ½ second time intervals. At the 10-minute mark (end of baseline), ultrasound treatment began. An Omnisound 3000 (Accelerated
Care Plus, Reno, NV) ultrasound unit with a beam non-uniformity ratio of 4:1 for 3 MHz was used to deliver the ultrasound treatment. The intensity was set at 1.0 W/cm² on a continuous beam cycle. The 5 cm² ultrasound (transducer) head was moved in longitudinal stokes within the template at a rate of 4 cm/sec using a digital metronome (Seiko S-Yard, Inc., China). The ultrasound treatment continued until the absolute temperature increased 3.0°C above the 10-minute baseline measure. Three degrees above baseline was selected as the target temperature for absolute depth based upon the ability of the temperature to decrease muscle spasms and pain and increase blood flow and metabolic cellular rates (Lehman et al., 1967a, 1967b). Treatment was then stopped and temperature was recorded for an additional 20 minutes, serving as the recovery period. The gel remained on the treatment site for the entire baseline, treatment, and recovery periods.

Upon completion of the 20-minute recovery period, temperature recordings were ceased. Excess gel was removed using paper towels. The site was then cleansed again with 70% isopropyl alcohol pads. The absolute thermocouple was pulled out first followed by the relative thermocouple. Pressure with a 70% isopropyl alcohol pad was placed over the two thermocouple sites for approximately 20 seconds after being pulled out of the calf. A Band-Aid was placed over the two thermocouple sites. The surface temperature wire was then removed. The subjects were instructed to stretch and ice the calf 2–3 times during the remainder of the day and examine the site for possible infection. An ice bag was given to subjects when they left the laboratory.
Statistical Analysis

To examine research question 1 (RQ1)—What are the absolute and relative intramuscular and skin temperatures and overall rate of heating per minute for each, respectively, during 3 MHz ultrasound treatment?—mean scores for absolute, relative, and skin temperature for the ultrasound treatment time and heating rates per unit of time dependent upon degree (1°C, 2°C, and 3°C) above baseline were calculated.

To examine research question 2 (RQ2)—Does skin temperature predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?—three separate analytical techniques, OLS regression, HLM, and MMRM, were used to determine how skin temperature predicted absolute and relative temperatures during the 3 MHz ultrasound treatment.

To examine research question 3 (RQ3)—Does subcutaneous skinfold thickness predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?—three separate analytical techniques, OLS regression, HLM, and MMRM, were used to determine how skinfold thickness predicted absolute and relative temperatures during the 3 MHz ultrasound treatment.

To examine research question 4 (RQ4)—Do the three analysis models (OLS regression, HLM, or MMRM) produce similar outcomes for the research questions?—comparisons of the results and examination of normality were discussed with the reporting of research questions 2 and 3.

All descriptive data and OLS regression were analyzed using SPSS (version 17.0, Chicago, IL). HLM analysis was conducted using a beta version HLM2 (6.31) program.
with robust estimation techniques. MMRM analysis was conducted using SAS 9.1.3 (Cary, NC). All significance tests were set *a priori* at $p < .05$. 
CHAPTER IV

RESULTS

There are several purposes of this study expressed in four research questions:

RQ1. What are the absolute and relative intramuscular and skin temperatures and overall rate of heating per minute for each, respectively, during 3 MHz ultrasound treatment?

RQ2. Does skin temperature predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?

RQ3. Does subcutaneous skinfold thickness predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?

RQ4. Do the three analysis models (OLS regression, HLM, or MMRM) produce similar outcomes for the research questions?

This chapter presents the statistical analysis and related results for these RQs by question. The results are organized into several sections: (1) subject and variable characteristics; (2) ultrasound baseline and treatment temperatures; (3) absolute, relative, and skin temperature heating rates; (4) OLS regression, HLM, and MMRM analysis for ultrasound absolute and relative temperatures with skin temperature as a predictor; and (5) OLS regression, HLM, and MMRM analysis for ultrasound absolute and relative temperature with skinfold thickness as a predictor. Model comparisons are reported in Chapter V.
Subject and Variable Characteristics

A total of 33 subjects participated in the study. One subject was excluded during data analysis for erratic high temperature readings during the recovery period. This subject was excluded because the faulty readings in the recovery period could not be determined as an isolated event just to the recovery period, which may have compromised the temperature recordings during the baseline and treatment periods. A final total of 32 subjects was included for the data analysis. There were 14 males and 18 females with an average age of 22.7 ± 1.6 years. All subject demographic variables are summarized in Table 2.

Table 2

Subject Demographics (N = 32)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Min.</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht (cm)</td>
<td>152.4</td>
<td>198.1</td>
<td>174.8</td>
<td>11.9</td>
</tr>
<tr>
<td>Wt (kgs)</td>
<td>49.0</td>
<td>127.0</td>
<td>76.2</td>
<td>18.2</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>20.0</td>
<td>26.0</td>
<td>22.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Skinfold (mm)</td>
<td>8.0</td>
<td>26.0</td>
<td>15.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Calfcir (mm)</td>
<td>31.0</td>
<td>42.5</td>
<td>36.8</td>
<td>2.8</td>
</tr>
<tr>
<td>BMI</td>
<td>17.8</td>
<td>35.9</td>
<td>24.8</td>
<td>4.7</td>
</tr>
</tbody>
</table>
To determine the relationships between demographic and treatment variables, Pearson product-moment correlations were calculated (Table 3). The correlations were based upon the ending ultrasound treatment of a 3°C rise from baseline temperature recorded from the thermocouple inserted at the absolute depth. Results show that weight ($r = -.43$) and height ($r = -.35$) were inversely correlated with absolute ending treatment temperature.

Table 3

*Demographic and Treatment Temperature Correlations (N = 32)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Skin Temp</th>
<th>Absol Temp</th>
<th>Rel Temp</th>
<th>Total Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skinfold</td>
<td>-.09</td>
<td>.26</td>
<td>.02</td>
<td>.06</td>
</tr>
<tr>
<td>(2-tailed sig.)</td>
<td>.63</td>
<td>.15</td>
<td>.92</td>
<td>.73</td>
</tr>
<tr>
<td>CalfCir</td>
<td>-.23</td>
<td>-.13</td>
<td>-.14</td>
<td>-.02</td>
</tr>
<tr>
<td>(2-tailed sig.)</td>
<td>.20</td>
<td>.48</td>
<td>.44</td>
<td>.92</td>
</tr>
<tr>
<td>Weight</td>
<td>-.14</td>
<td>-.43</td>
<td>-.20</td>
<td>-.09</td>
</tr>
<tr>
<td>(2-tailed sig.)</td>
<td>.45</td>
<td>.02</td>
<td>.26</td>
<td>.59</td>
</tr>
<tr>
<td>Height</td>
<td>-.02</td>
<td>-.35</td>
<td>-.22</td>
<td>-.25</td>
</tr>
<tr>
<td>(2-tailed sig.)</td>
<td>.91</td>
<td>.05</td>
<td>.22</td>
<td>.18</td>
</tr>
<tr>
<td>BMI</td>
<td>-.16</td>
<td>-.23</td>
<td>-.08</td>
<td>.05</td>
</tr>
<tr>
<td>(2-tailed sig.)</td>
<td>.39</td>
<td>.20</td>
<td>.68</td>
<td>.80</td>
</tr>
</tbody>
</table>

Ultrasound Treatment Temperatures

The ultrasound treatment period was defined as a 3°C increase in absolute temperature from the ending of the baseline period. The ultrasound mean ending
treatment temperatures for skin, absolute, and relative plus the overall mean ending
treatment time for all subjects from baseline are presented in Table 4 and Figures 4–6. As
seen in the figures, the initial temperature values and ending values varied by person.
These values should vary since temperature per person is based upon multiple factors,
such as lean body mass and metabolic rates. Skin temperature increased 10.9°C, while
relative temperature increased from baseline 2.1°C over the treatment period. The mean
ending time to reach 3°C above baseline was approximately 294.3.

Table 4

Ultrasound Ending Temperatures and Time (N = 32)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Min.</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>24.3</td>
<td>28.6</td>
<td>26.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Absolute</td>
<td>33.0</td>
<td>36.7</td>
<td>35.2</td>
<td>.85</td>
</tr>
<tr>
<td>Relative</td>
<td>32.6</td>
<td>36.5</td>
<td>35.5</td>
<td>.79</td>
</tr>
<tr>
<td>End of Ultrasound (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>33.0</td>
<td>43.6</td>
<td>37.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Absolute</td>
<td>36.0</td>
<td>39.7</td>
<td>38.2</td>
<td>.85</td>
</tr>
<tr>
<td>Relative</td>
<td>34.6</td>
<td>39.8</td>
<td>37.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Time to ABS + 3°C (secs)</td>
<td>20.0</td>
<td>26.0</td>
<td>22.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>
Figure 4. Skin Temperatures for All Subjects

Figure 5. Absolute Temperatures for All Subjects
RQ1 – Heating Rates

Increases of temperature from baseline ending values to 3°C above for absolute temperature were tabulated by degree intervals, from 1°C to 3°C, and the corresponding time to achieve these respective temperatures was averaged for all subjects. The mean time for absolute temperature to reach 1°C above baseline was 70.96 seconds, 167.34 seconds for 2°C above baseline, and 294.25 seconds for 3°C above baseline. The average rate of increase per minute for each degree change was .84°C, .71°C, and .61°C, respectively, indicating that temperature rose in a curvilinear manner with a higher heating rate at the beginning of the treatment compared to the middle and ending of the treatment period, which is reflected in Figures 4–6. The same curvilinear temperature rise was observed at the skin and relative recording sites (Table 5).
Table 5

*Site, Temperature, and Rate of Change by Degree (N = 32)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time</th>
<th>Mean</th>
<th>SD</th>
<th>Rate (Min/Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0</td>
<td>26.38</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>1°C</td>
<td>70.96</td>
<td>30.61</td>
<td>1.65</td>
<td>3.57°C/.049°C</td>
</tr>
<tr>
<td>2°C</td>
<td>167.34</td>
<td>34.19</td>
<td>2.44</td>
<td>2.80°C/.046°C</td>
</tr>
<tr>
<td>3°C</td>
<td>294.25</td>
<td>37.24</td>
<td>2.78</td>
<td>2.21°C/.036°C</td>
</tr>
<tr>
<td>Absolute</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0</td>
<td>35.15</td>
<td>.84</td>
<td>.84°C/.014°C</td>
</tr>
<tr>
<td>1°C</td>
<td>70.96</td>
<td>36.15</td>
<td>.84</td>
<td>.71°C/.011°C</td>
</tr>
<tr>
<td>2°C</td>
<td>167.34</td>
<td>37.15</td>
<td>.85</td>
<td>.61°C/.010°C</td>
</tr>
<tr>
<td>3°C</td>
<td>294.25</td>
<td>38.15</td>
<td>.85</td>
<td>.61°C/.010°C</td>
</tr>
<tr>
<td>Relative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0</td>
<td>35.52</td>
<td>.81</td>
<td></td>
</tr>
<tr>
<td>1°C</td>
<td>70.96</td>
<td>36.13</td>
<td>.86</td>
<td>.51°C/.008°C</td>
</tr>
<tr>
<td>2°C</td>
<td>167.34</td>
<td>36.85</td>
<td>.99</td>
<td>.47°C/.0079°C</td>
</tr>
<tr>
<td>3°C</td>
<td>294.25</td>
<td>37.61</td>
<td>1.21</td>
<td>.42°C/.0071°C</td>
</tr>
</tbody>
</table>

* The time variable was based upon the absolute time change from baseline to each degree, respectively.

**RQ2 – Absolute and Skin Temperature**

*OLS Regression Analysis for Absolute Temperature and Skin Temperature*

OLS regression was performed between the dependent variable of absolute temperature and the independent variable of time and skin temperature (Table 6). Time was included in the model since the treatment was time dependent. A Pearson product-moment correlation coefficient between time (.360) and skin temperature (.295) showed
some relationship with absolute temperature. Multicollinearity was checked with Tolerance and VIF, which were .548 and 1.825, respectively, suggesting that the correlations between the independent variables were low. Outliers were checked using the Mahalanobis distances with a critical chi-square value of 13.82 based upon two independent variables. Results indicated the lack of outliers of the data. Cooks distances maximum value of .238 revealed no subject was beyond limits of 1.0 (Pallant, 2006).

Table 6

*Absolute Regression Model With Time and Skin Temperature*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B$</td>
<td>Std Error</td>
</tr>
<tr>
<td>Constant</td>
<td>36.335</td>
<td>2.322</td>
</tr>
<tr>
<td>Time</td>
<td>.002</td>
<td>.002</td>
</tr>
<tr>
<td>Skin Temperature</td>
<td>.030</td>
<td>.071</td>
</tr>
</tbody>
</table>

The regression analysis revealed that the overall model was not significant, $F(2, 31) = 2.26, p = .123$, with an $R^2$ of .135. Partial correlations show that time explained .218 (4.7%) and skin temperature .072 (.05%) of the variance in absolute temperature in the model. The constant term was 36.335 with an unstandardized coefficient for time at .002 and skin temperature at .030; therefore, the prediction equation produced was $Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon$, where $Y$ is the response variable, $\beta_0$ is the intercept, $\beta_1 =$ coefficient for time, $x_1 =$ predictor variable measured in seconds, $\beta_2 =$ coefficient for skin temperature, $x_2 =$ predictor variable measured in degrees, and $\varepsilon =$ error term:

Absolute temperature = 36.33 + .002 * (time in seconds) + .030 * (degrees) + $\varepsilon$
**Hierarchical Linear Modeling for Absolute Temperature**

HLM was used to examine the within-person temperature variations during the ultrasound treatment time. Time was defined as the time at the beginning of the treatment (zero seconds) and concluded when the temperature for absolute changed 3°C, which varied for each person. The time component must first be included in the model before the addition of skin temperature in order to explain the unique contribution of the skin temperature variable. The inclusion of time allows the data to examine the individual growth curves. The level 1 predictor was uncentered. The level 1 model, which represents the repeated measures of time nested within persons is as follows:

**Level 1 model**:

\[ Y_{ti} = \pi_{0i} + \pi_{1i} \text{(time)} + e_{ti}, \text{ with } e_{ti} \sim N(0, \sigma^2) \]

Where \( Y_{ti} \) is the absolute temperature for person \( i \) at time \( t \)

\( \pi_{0i} \) is the baseline temperature for person \( i \)

\( \pi_{1i} \) is the linear growth rate

\( e_{ti} \) is the random error within person

\( \sigma^2 \) is the level 1 variance

**Level 2 model**:

\[ \pi_{0i} = \beta_{00} + r_{0i} \]

\[ \pi_{1i} = \beta_{10} + r_{1i} \]

Where \( \beta_{00} \) is the mean baseline temperature across all individuals

\( \beta_{10} \) is the mean growth rate across persons

\( r_{0i} \) is the random error associated with mean baseline temperature

\( r_{1i} \) is the random error associated with the growth rate
Written as a combined model formula:

\[ Y_{it} = \beta_{00} + \beta_{10} \text{(time)} + r_{0i} + r_{1i} \text{(time)} + e_{it} \]

As expected, the results indicated a significant linear growth rate (Table 7). The constant term was 35.39 with a coefficient for time at .0107.

Table 7

*HLM for Absolute Temperature and Time*

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>Coefficient</th>
<th>df</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta_{00} ) (mean baseline temp)</td>
<td>35.39</td>
<td>31</td>
<td>.15</td>
<td>235.29*</td>
</tr>
<tr>
<td>( \beta_{10} ) (mean growth rate)</td>
<td>.0107</td>
<td>31</td>
<td>.0006</td>
<td>17.94*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random Effect</th>
<th>Variance Component</th>
<th>df</th>
<th>Std Dev</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_{0i} ) (error baseline)</td>
<td>.7475</td>
<td>31</td>
<td>.86</td>
<td>248108.83*</td>
</tr>
<tr>
<td>( r_{1i} ) (error growth rate)</td>
<td>.00001</td>
<td>31</td>
<td>.003</td>
<td>243297.67*</td>
</tr>
<tr>
<td>( e_{it} )</td>
<td>.0126</td>
<td></td>
<td>.112</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at \( p < .05 \).

*Hierarchical Linear Modeling for Absolute Temperature and Skin Temperature*

The next step in building the HLM model was adding the independent variable of skin temperature. The variable of skin temperature, a time varying covariate, was added in the level 1 model because the skin temperature changes within each person as a
function of the ultrasound treatment. The skin temperature variable was centered around the grand mean. The new level 1 and 2 models are:

Level 1: \( Y_{it} = \pi_{0i} + \pi_{1i} (\text{skin}) + \pi_{2i} (\text{time}) + e_{it}, \text{ with } e_{it} \sim N(0, \sigma^2) \) \[3\]

Where \( Y_{it} \) is the absolute temperature for person \( i \) at time \( t \)

\( \pi_{0i} \) is the baseline temperature for person \( i \) at baseline adjusted for skin temperature

\( \pi_{1i} \) is the effect of skin temperature

\( \pi_{2i} \) is the linear growth parameter for each person

\( e_{it} \) is the random error within persons

\( \sigma^2 \) is the adjusted level 1 variance

Level 2 model: \( \pi_{0i} = \beta_{00} + r_{0i} \) \[4\]

\( \pi_{1i} = \beta_{10} + r_{1i} \)

\( \pi_{2i} = \beta_{20} + r_{2i} \)

Where \( \beta_{00} \) is the mean baseline temperature across all individuals

\( \beta_{10} \) is the mean effect of skin temperature

\( \beta_{20} \) is the mean linear growth rate across all individuals

\( r_{0i} \) is the random error associated with mean baseline temperature

\( r_{1i} \) is the random error associated with the mean effect of skin temperature

\( r_{2i} \) is the random error associated with growth rate

Written as a combined model formula:

\( Y_{it} = \beta_{00} + \beta_{10} (\text{skin}) + \beta_{20} (\text{time}) + r_{0i} + r_{1i} (\text{skin}) + r_{2i} (\text{time}) + e_{it} \)
The findings suggest that the growth parameter \((0.0077, p < 0.001)\) and skin temperature \((0.0853, p < 0.001)\) were significant predictors in the model (Table 8). That is, as skin temperature increased, absolute temperature increased over the course of the ultrasound treatment. In addition, growth rates varied among persons \((r_{2i} = 0.00002, p < 0.001)\) suggesting that persons had varying temperatures during the ultrasound treatment.

Table 8

*HLM for Skin Temperature and Time on Absolute Temperature*

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>Coefficient</th>
<th>df</th>
<th>SE</th>
<th>(t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta_{00}) (mean baseline temp)</td>
<td>35.85</td>
<td>31</td>
<td>.17</td>
<td>209.96*</td>
</tr>
<tr>
<td>(\beta_{10}) (mean effect of skin)</td>
<td>.08</td>
<td>31</td>
<td>.013</td>
<td>6.63*</td>
</tr>
<tr>
<td>(\beta_{20}) (mean growth rate)</td>
<td>.007</td>
<td>31</td>
<td>.0007</td>
<td>10.38*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random Effect</th>
<th>Variance Component</th>
<th>df</th>
<th>Std Dev</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(r_{0i}) (error baseline)</td>
<td>.9611</td>
<td>6</td>
<td>.98</td>
<td>12680.53*</td>
</tr>
<tr>
<td>(r_{1i}) (error of skin)</td>
<td>.0054</td>
<td>6</td>
<td>.0735</td>
<td>1588.24*</td>
</tr>
<tr>
<td>(r_{2i}) (error growth rate)</td>
<td>.00002</td>
<td>6</td>
<td>.0043</td>
<td>6950.28*</td>
</tr>
<tr>
<td>(e_{ni})</td>
<td>.0059</td>
<td></td>
<td>.077</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at \(p < .05\).

The variance component of this model was compared to an unconditional model (model that did not include level 1 or level 2 variables). The unconditional model
produced a between-person baseline temperature variance at .7481, while the variance associated with the absolute temperature model in this section \( r_{0i} \) was .9611. Therefore, the addition of the skin temperature and time variables produced approximately 28% more of the between-person’s baseline temperature variability of the unconditional model.

**Mixed Model Repeated Measures for Absolute Temperature and Skin Temperature**

A non-centered repeated measures model for the response variable absolute temperature (°C) regressed onto skin temperature (°C) for the treatment period was analyzed. The time variable, which varies per person, was built into the model for analysis with the overall time per subject divided into 10 parts over the ultrasound treatment. Thus, time is represented in the output as the changes in degrees over the ultrasound treatment period by the predictor variable, skin temperature.

Results demonstrated that skin temperature was not predictive of absolute temperature for the treatment period \((p = 0.586)\) after modeling the repeated effect time with the Compound-Symmetric variance-covariance matrix (Table 9). The prediction equation produced was \( Y = \beta_0 + \beta_1 x_1 + \varepsilon \), where \( Y \) is the response variable, \( \beta_0 \) is the intercept, \( \beta_1 \) = coefficient for skin temperature, \( x_1 \) = predictor variable measured degrees, and \( \varepsilon \) = error term: Absolute temperature = 37.88 + .002 * (degrees) + \( \varepsilon \)

Further investigation of this model revealed that the residuals were non-normal \((Shapiro-Wilk p < 0.001)\). As a result, the above model was repeated with a \( \log_e \) and rank transformation of the response variable. Both of these models confirm skin temperature to not be predictive of absolute temperature \((\log_e: 0.872; \text{rank: 0.820})\).
Table 9

**MMRM for the Predictor of Skin Temperature on Absolute Treatment Temperature**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Std Err</th>
<th>df</th>
<th>t</th>
<th>p</th>
<th>CI Lower</th>
<th>CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>37.88</td>
<td>.24</td>
<td>143</td>
<td>160.74</td>
<td>&lt;.0001</td>
<td>37.41</td>
<td>38.38</td>
</tr>
<tr>
<td>Mskin</td>
<td>.002</td>
<td>.005</td>
<td>280</td>
<td>.55</td>
<td>&lt;.586</td>
<td>−.007</td>
<td>.012</td>
</tr>
</tbody>
</table>

*Note.* Mskin = mean skin temperature.

**RQ2 – Relative and Skin Temperature**

**OLS Regression Analysis for Relative Temperature and Skin Temperature**

An OLS regression was performed between the dependent variable of relative treatment temperature and the independent variable of time and skin temperature (Table 10). Time was included in the model since the treatment was time dependent. The Pearson product-moment correlation coefficients between time (.609) and skin temperature (.492) showed some relationship with relative temperature. Multicollinearity was checked with Tolerance and VIF and outliers were checked using the Mahalanobis distances with a critical chi-square value of 13.82 based upon the time and skin temperature variables. Results indicated a lack of outliers in the data. Cooks distances maximum value of .210 revealed no subject was beyond limits of 1.0 (Pallant, 2006).

The regression analysis revealed that the overall model was significant, $F(2, 31) = 9.005, p = .001$, with an $R^2$ of .383. Partial correlations show that time explained .376 (14%) and skin temperature .111 (1%) of the variance in relative temperature in the model. Time was the significant predictor, but skin temperature was not a significant
predictor in the model. The constant term was 33.42 with an unstandardized coefficient for time at .006 and skin temperature at .065; therefore, the prediction equation produced was $Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \epsilon$, where $Y$ is the response variable, $\beta_0$ is the intercept, $\beta_1 =$ coefficient for time, $x_1 =$ predictor variable measured in seconds, $\beta_2 =$ coefficient for skin temperature, $x_2 =$ predictor variable measured in degrees, and $\epsilon =$ error term:

Relative temperature = 33.42 + .006 * (time in seconds) + .065 * (degrees) + \epsilon

Table 10

**Relative Regression Model With Time and Skin Temperature**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B$</td>
<td>Std Error</td>
</tr>
<tr>
<td>Constant</td>
<td>33.424</td>
<td>2.781</td>
</tr>
<tr>
<td>Time</td>
<td>.006</td>
<td>.002</td>
</tr>
<tr>
<td>Skin Temperature</td>
<td>.065</td>
<td>.085</td>
</tr>
</tbody>
</table>

**Hierarchical Linear Modeling for Relative Temperature and Time**

HLM was used to examine the within-person temperature variations during the ultrasound treatment time period. The time component must first be included in the model before the addition of skin temperature in order to explain the unique contribution of the skin temperature variable. The inclusion of time allows the data to examine the individual growth curves. The level 1 predictor was uncentered. The level 1 model represents the repeated measures of time nested within persons. The level 2 model was
previously presented in equation 2 and will be presented here again but with definition changes of the variables. The models are as follows:

**Level 1 model:**

\[ Y_i(t) = \pi_{0i} + \pi_1(t) + e_{it}, \]

where \( Y_i(t) \) is the relative temperature for person \( i \) at time \( t \)

- \( \pi_{0i} \) is the baseline temperature for person \( i \)
- \( \pi_1(t) \) is the linear growth rate
- \( e_{it} \) is the random error within person
- \( \sigma^2 \) is the level 1 variance

**Level 2 model:**

\[ \pi_{0i} = \beta_{00} + r_{0i}, \quad \pi_{1i} = \beta_{10} + r_{1i}, \]

where \( \beta_{00} \) is the mean baseline temperature across all individuals

- \( \beta_{10} \) is the mean growth rate across persons
- \( r_{0i} \) is the random error associated with mean baseline temperature
- \( r_{1i} \) is the random error associated with the growth rate

Written as a linear growth model formula:

\[ Y_i(t) = \beta_{00} + \beta_{10} \text{ (time)} + r_{0i} + r_{1i} \text{ (time)} + e_{it} \]

The findings suggest that the growth rate was significant \((p < .05)\) (see Table 11). The constant term was 35.62 with a coefficient for growth at .007. In addition, the growth rates varied across persons \((r_{2i} = 0.00001, p < 0.001)\) suggesting that persons had varying relative temperatures during the ultrasound treatment.
Table 11

*HLM for Relative Temperature and Time*

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>Coefficient</th>
<th>df</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_{00}$ (mean baseline temp)</td>
<td>35.62</td>
<td>31</td>
<td>.14</td>
<td>253.93*</td>
</tr>
<tr>
<td>$\beta_{10}$ (mean growth rate)</td>
<td>.007</td>
<td>31</td>
<td>.0005</td>
<td>15.17*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random Effect</th>
<th>Variance Component</th>
<th>df</th>
<th>Std Dev</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{0i}$ (error baseline)</td>
<td>.6503</td>
<td>31</td>
<td>.81</td>
<td>393168.81*</td>
</tr>
<tr>
<td>$r_{1i}$ (error growth rate)</td>
<td>.00001</td>
<td>31</td>
<td>.003</td>
<td>129471.46*</td>
</tr>
<tr>
<td>$e_n$</td>
<td>.0063</td>
<td></td>
<td>.079</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at $p < .05$.

Hierarchical Linear Modeling for Relative Temperature With Time and Skin Temperature

The next step in building the HLM model was adding the independent variable of skin temperature. The variable of skin temperature, a time varying covariate, was added because the skin temperature changes within each person as a function of the ultrasound treatment. The skin temperature variable was centered around the grand mean. The level 2 model was previously presented in equation 4 and will be presented here again but with definition changes of the variables. The models are as follows:

Level 1: $Y_{ni} = \pi_{0i} + \pi_{1i} \text{ (skin)} + \pi_{2i} \text{ (time)} + e_{ni}$, with $e_{ni} \sim N(0, \sigma^2)$  \[6\]

Where $Y_{ni}$ is the relative temperature for person $i$ at time $t$
\( \pi_{0i} \) is the baseline temperature for person \( i \) at baseline adjusted for skin temperature

\( \pi_{1i} \) is the effect of skin temperature

\( \pi_{2i} \) is the linear growth parameter for each person

\( e_{ii} \) is the random error within persons

\( \sigma^2 \) is the adjusted level 1 variance

Level 2 model: 
\[
\pi_{0i} = \beta_{00} + r_{0i} \\
\pi_{1i} = \beta_{10} + r_{1i} \\
\pi_{2i} = \beta_{20} + r_{2i}
\]

Where 
\( \beta_{00} \) is the mean baseline temperature across all persons

\( \beta_{10} \) is the mean effect of skin temperature

\( \beta_{20} \) is the mean linear growth rate across all persons

\( r_{0i} \) is the random error associated with mean baseline temperature

\( r_{1i} \) is the random error associated with the mean effect of skin temperature

\( r_{2i} \) is the random error associated with growth rate

Written as a combined model formula:
\[
Y_{ii} = \beta_{00} + \beta_{10} \text{ (skin)} + \beta_{20} \text{ (time)} + r_{0i} + r_{1i} \text{ (skin)} + r_{2i} \text{ (time)} + e_{ii}
\]

The findings suggest that the growth parameter (0.006, \( p < 0.001 \)) and skin temperature (0.044, \( p < 0.001 \)) were significant predictors in the model (Table 12). As the skin temperature increased, the relative temperature increased over the course of the ultrasound treatment. In addition, the growth rates vary across persons (\( r_{2i} = 0.00001 \),
suggesting that persons had varying temperature rates during the ultrasound treatment.

The variance component of this model was compared to an unconditional model (model that did not include level 1 or level 2 variables). The unconditional model produced a between-person baseline temperature variance at .9343, while the variance associated with the relative temperature model ($r_{0i}$) was .8199. Therefore, the addition of the skin temperature and time variables explained approximately 12% of the between-person’s baseline temperature variability of the unconditional model.

Table 12

*HLM* for Skin Temperature and Time on Relative Temperature

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>Coefficient</th>
<th>df</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_{00}$ (mean baseline temp)</td>
<td>35.89</td>
<td>31</td>
<td>.16</td>
<td>227.61*</td>
</tr>
<tr>
<td>$\beta_{10}$ (mean effect of skin)</td>
<td>.044</td>
<td>31</td>
<td>.011</td>
<td>4.21*</td>
</tr>
<tr>
<td>$B_{20}$ (mean growth rate)</td>
<td>.006</td>
<td>31</td>
<td>.0005</td>
<td>11.08*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random Effect</th>
<th>Variance Component</th>
<th>df</th>
<th>Std Dev</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{00}$ (error baseline)</td>
<td>.8199</td>
<td>6</td>
<td>.9055</td>
<td>15556.15*</td>
</tr>
<tr>
<td>$r_{1i}$ (error of skin)</td>
<td>.0036</td>
<td>6</td>
<td>.0602</td>
<td>1987.74*</td>
</tr>
<tr>
<td>$r_{2i}$ (error growth rate)</td>
<td>.00001</td>
<td>6</td>
<td>.0029</td>
<td>6111.23*</td>
</tr>
<tr>
<td>$e_n$</td>
<td>.0035</td>
<td></td>
<td>.0598</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at $p < .05$. 
Mixed Model Repeated Measures for Relative Temperature and Skin Temperature

A non-centered repeated measures model for the response variable relative temperature (°C) regressed onto skin temperature (°C) for the treatment period was analyzed. The time variable, which varies per person, was built into the model for analysis with the overall time per subject divided into 10 parts over the ultrasound treatment, as stated previously.

Results (raw model) demonstrated that skin temperature was predictive of relative temperature for the treatment period \((p < 0.0001)\) after modeling the repeated effect time with the Compound-Symmetric variance-covariance matrix (Table 13). The prediction equation produced was \(Y = \beta_0 + \beta_1 x_1 + \epsilon\), where \(Y\) is the response variable, \(\beta_0\) is the intercept, \(\beta_1 = \) coefficient for skin temperature, \(x_1 = \) predictor variable measured degrees, and \(\epsilon = \) error term: Relative temperature = 32.61 + .133 * (degrees) + \(\epsilon\).

Table 13

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Std Err</th>
<th>df</th>
<th>(t)</th>
<th>(p)</th>
<th>CI Lower</th>
<th>CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>32.61</td>
<td>.62</td>
<td>309</td>
<td>51.97</td>
<td>&lt;.0001</td>
<td>31.37</td>
<td>33.84</td>
</tr>
</tbody>
</table>
| Ms
| .133 | .016 | 299 | 8.10 | <.0001 | .10 | .16 |

Note. Ms = mean skin temperature.

Further investigation of this model revealed that the residuals were non-normal (Shapiro-Wilk \(p < 0.001\)). As a result, the above model was repeated with a loge and rank
transformation of the response variable. Both of these models confirm skin temperature to be predictive of relative temperature ($\log_{e}: <0.0001$; rank: $<0.0001$).

RQ3 – Absolute and Skinfold Thickness

**OLS Regression Analysis for Absolute Temperature With Skinfold Thickness**

OLS regression was performed between the dependent variable of absolute temperature and the independent variables of time and skinfold thickness (Table 14). Time was included in the model since the treatment was time dependent. The Pearson product-moment correlation coefficients between time (.360) and skinfold thickness (.261) show some relationship with absolute temperature. Multicollinearity was checked with Tolerance and VIF, which were .996 and 1.004, respectively, for both independent variables, suggesting that correlation between the independent variables were low. Outliers were checked using the Mahalanobis distances with a critical chi-square value of 13.82 based upon two independent variables. Results indicated the lack of outliers of the data. Cooks distances maximum value of .258 revealed no subject was beyond limits of 1.0 (Pallant, 2006).

The regression analysis revealed that the overall model was a significant predictor of absolute temperature, $F(2, 31) = 3.33, p = .050$, with an $R^2$ of .187. Partial correlations showed that time explained .344 (11.8%) and skinfold thickness explained .239 (5.7%) of the variance in absolute temperature in the model. The constant term was 36.66 with an unstandardized coefficient for time at .003 and skinfold thickness at .042; therefore, the prediction equation produced was $Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon$, where $Y$ is the response
variable, $\beta_0$ is the intercept, $\beta_1 = \text{coefficient for time}$, $x_1 = \text{predictor variable measured in seconds}$, $\beta_2 = \text{coefficient for skinfold}$, $x_2 = \text{predictor variable measured in millimeters}$, and $\epsilon = \text{error term}$:

$$\text{Absolute temperature} = 36.66 + .003 \times (\text{time in seconds}) + .042 \times (\text{skinfold in millimeters}) + \epsilon$$

Upon further examination of the model, only the time variable was significant ($p < .05$), not skinfold thickness ($p = .164$), as a predictor in the model.

Table 14

*Absolute Regression Model With Time and Skinfold Thickness*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B$</td>
<td>Std Error</td>
</tr>
<tr>
<td>Constant</td>
<td>36.66</td>
<td>.616</td>
</tr>
<tr>
<td>Time</td>
<td>.003</td>
<td>.001</td>
</tr>
<tr>
<td>Skinfold Thickness</td>
<td>.042</td>
<td>.03</td>
</tr>
</tbody>
</table>

*Hierarchical Linear Modeling for Absolute Temperature With Time and Skinfold Thickness*

HLM was used to examine the within-person temperature variations during the ultrasound treatment time period. Time was defined as the time at the beginning of the treatment (zero seconds) and concluded when the temperature for absolute changed 3°C. The ending time varied upon person since the time to reach 3°C above baseline varied upon person. The time component must first be included in the model before the addition of skin temperature in order to explain the unique contribution of the skin temperature
variable. The inclusion of time allows the examination of individual growth curves. Also included is the variable of skinfold thickness as a level 2 predictor. The level 1 predictor (time) was uncentered, while skin temperature and the level 2 predictor were grand mean centered. The level 1 model was presented previously as equation 3. The new level 2 model is:

\[ \pi_{oi} = \beta_{00} + \beta_{01} \text{ (skinfold)} + r_{0i}, \]  

\[ \pi_{ti} = \beta_{10} + \beta_{11} \text{ (skinfold)} + r_{1i}, \]  

Where \( \beta_{00} \) is the mean baseline temperature across persons  
\( \beta_{01} \) is the effect of skinfold thickness on baseline temperature  
\( \beta_{10} \) is the mean growth rate across  
\( \beta_{11} \) is the effect of skinfold thickness on the growth rate  
\( r_{0i} \) is the random error associated with baseline temperature  
\( r_{1i} \) is the random error associated with growth rates

Written as a linear growth model formula:

\[ Y_{ti} = \beta_{00} (\text{temp}) + \beta_{01} (\text{skin}) + \beta_{10} (\text{time}) + \beta_{11} (\text{skin}) + r_{0i} (\text{temp/skin}) + r_{1i} (\text{time/skin}) + e_{ti}. \]

The findings suggest that the growth parameter (.0108, \( p < 0.001 \)) and not skinfold thickness (−.00009, \( p = .471 \)) was a significant predictor in the model (Table 15). Therefore, adding the skinfold thickness variable to the equation did not influence the model. In addition, growth rates varied among persons (\( r_{1i} = 0.00001, p < 0.001 \)) suggesting that persons had varying temperatures during the ultrasound treatment.
The variance component of this model was compared to an unconditional model (model that did not include level 1 or level 2 variables). The unconditional model produced a between person baseline temperature variance at .7481, while the variance associated with the absolute temperature model \( (r_{0i}) \) was .7196. Therefore, the addition of the time and skinfold variables explained approximately 4% of the between-person’s baseline temperature variability of the unconditional model.

Table 15

*HLM for Time as Level 1 Covariate and Skinfold Thickness as a Level 2 Covariate*

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>Coefficient</th>
<th>df</th>
<th>SE</th>
<th>( t )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta_{00} ) (mean baseline temp)</td>
<td>35.89</td>
<td>30</td>
<td>.1452</td>
<td>243.75*</td>
</tr>
<tr>
<td>( \beta_{01} ) (effect of skinfold mean)</td>
<td>.0467</td>
<td>30</td>
<td>.0269</td>
<td>1.73</td>
</tr>
<tr>
<td>( \beta_{10} ) (mean growth rate)</td>
<td>.0108</td>
<td>30</td>
<td>.0006</td>
<td>18.09*</td>
</tr>
<tr>
<td>( B_{11} ) (effect of growth rate)</td>
<td>−.00009</td>
<td>30</td>
<td>.0001</td>
<td>−.729</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random Effect</th>
<th>Variance Component</th>
<th>df</th>
<th>Std Dev</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_{0i} ) (error baseline)</td>
<td>.7196</td>
<td>30</td>
<td>.8483</td>
<td>225392.07*</td>
</tr>
<tr>
<td>( r_{1i} ) (error growth)</td>
<td>.00001</td>
<td>30</td>
<td>.0035</td>
<td>246226.76*</td>
</tr>
<tr>
<td>( e_{ii} )</td>
<td>.0126</td>
<td>.1121</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at \( p < .05 \).
Mixed Model Repeated Measures for Absolute Temperature and Skinfold Thickness

A non-centered repeated measures model for the response variable Absolute temperature (°C) regressed onto skinfold thickness (mm) for the treatment period was analyzed. Results demonstrate that skinfold thickness was not predictive of absolute temperature for the treatment period ($p = 0.154$) after modeling the repeated effect time with the Compound-Symmetric variance-covariance matrix (Table 16).

Table 16

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Std Err</th>
<th>df</th>
<th>$T$</th>
<th>$p$</th>
<th>CI Lower</th>
<th>CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>37.25</td>
<td>.51</td>
<td>30</td>
<td>71.67</td>
<td>&lt;.0001</td>
<td>36.19</td>
<td>38.31</td>
</tr>
<tr>
<td>MSkinfold</td>
<td>.044</td>
<td>.031</td>
<td>30</td>
<td>1.46</td>
<td>&lt;.154</td>
<td>-.01</td>
<td>.11</td>
</tr>
</tbody>
</table>

Note. MSkinfold = mean skinfold thickness.

The prediction equation produced was: $Y = \beta_0 + \beta_1 x_1 + \varepsilon$, where $Y$ is the response variable, $\beta_0$ is the intercept, $\beta_1$ = coefficient for skinfold, $x_1$ = predictor variable measured in millimeters, and $\varepsilon$ = error term:

Absolute temperature = 37.25 + .044 \times (skinfold thickness) + \varepsilon

Further investigation of this model revealed that the residuals were non-normal (Shapiro-Wilk $p < 0.001$). As a result, the above model was repeated with a $\log_e$ and rank transformation of the response variable. Both of these models confirm skinfold thickness was not predictive of absolute temperature ($\log_e$: 0.152; rank: 0.125).
RQ3 – Relative and Skinfold Thickness

**OLS Regression Analysis for Relative Temperature and Skinfold Thickness**

An OLS regression was performed between the dependent variable of relative treatment temperature and the independent variable of time and skinfold thickness (Table 17). Time was included in the model since the treatment was time dependent. The Pearson product-moment correlation coefficients between time (.609) and skinfold thickness (.017) show some relationship with relative temperature. Multicollinearity was checked with Tolerance and VIF and outliers were checked using the Mahalanobis distances with a critical chi-square value of 13.82 based upon the time and skin temperature variables. Results indicated a lack of outliers in the data. Cooks distances maximum value of .177 revealed no subject was beyond limits of 1.0 (Pallant, 2006).

**Table 17**

*Relative Regression Model With Time and Skinfold Thickness*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std Error</td>
</tr>
<tr>
<td>Constant</td>
<td>35.58</td>
<td>.769</td>
</tr>
<tr>
<td>Time</td>
<td>.007</td>
<td>.002</td>
</tr>
<tr>
<td>Skinfold Thickness</td>
<td>-.005</td>
<td>.037</td>
</tr>
</tbody>
</table>

The regression analysis revealed that the overall model was significant, $F(2, 31) = 8.558, \ p = .001$, with an $R^2$ of .371. The constant term was 35.58 with an unstandardized coefficient for time at .007 and skinfold thickness at -.005; therefore, the prediction
equation produced was $Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \epsilon$, where $Y$ is the response variable, $\beta_0$ is the intercept, $\beta_1$ = coefficient for time, $x_1$ = predictor variable measured in seconds, $\beta_2$ = coefficient for skinfold, $x_2$ = predictor variable measured in millimeters, and $\epsilon$ = error term:

Relative temperature $= 35.58 + .007 \times \text{(time in seconds)} - .005 \times \text{(skinfold in millimeters)} + \epsilon$

Upon further examination of the model, only the time variable was significant ($p < .05$), not skinfold thickness ($p = .887$), as a predictor in the model. Partial correlations showed that time explained .609 (37%) and skinfold thickness -.021 (.04%) of the variance of the relative temperature in the model.

*Hierarchical Linear Modeling for Relative Temperature, Time, and Skinfold Thickness*

HLM was used to examine the within-person temperature variation during the ultrasound treatment time period. Time was defined as the time at the beginning of the treatment (zero seconds) and concluded when the temperature for relative to reach $3^\circ$C. The ending time varied upon person since the time to reach $3^\circ$C above baseline varied upon person. Also included is the variable of skinfold thickness as a level 2 predictor. The level 1 predictor (time) was uncentered, while skin temperature and the level 2 predictor were grand mean centered. The level 1 model was presented previously as equation 6. The new level 2 model is:

Level 2 model:

\[
\pi_{0i} = \beta_{00} + \beta_{01} \text{(skinfold)} + r_{0i} \tag{8}
\]

\[
\pi_{1i} = \beta_{10} + \beta_{11} \text{(skinfold)} + r_{1i}
\]
Where $\beta_{00}$ is the mean baseline temperature across persons

$\beta_{01}$ is the effect of skinfold thickness on baseline temperature

$\beta_{10}$ is the mean growth rate across persons

$\beta_{11}$ is the effect of skinfold thickness on the growth rate

$r_{0i}$ is the random error associated with baseline temperature

$r_{1i}$ is the random error associated with growth rates

Written as a linear growth model formula:

$$ Y_{it} = \beta_{00} \text{ (temp)} + \beta_{01} \text{ (skin)} + \beta_{10} \text{ (time)} + \beta_{11} \text{ (skin)} + r_{0i} \text{ (temp/skin)} + r_{1i} \text{ (time/skin)} + e_{it} $$

The findings suggest that the growth parameter (.0072, $p < 0.001$) and skinfold thickness (-.0003, $p = .012$) were significant predictors in the model (Table 18). Therefore, adding the skinfold thickness variable to the equation influenced the model. The skinfold thickness was inversely proportional to the rise in relative temperature.

The variance component of this model was compared to an unconditional model (model that did not include level 1 or level 2 variables). The unconditional model produced a between person baseline temperature variance at .9343 while the variance associated with the relative temperature model ($r_{0i}$) was .6105. Therefore, the addition of the time and skinfold variables explained approximately 35% of the between person’s baseline temperature variability of the unconditional model.
Table 18

*HLM for Time as Level 1 Covariate and Skinfold Thickness as a Level 2 Covariate*

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>Coefficient</th>
<th>df</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_{00}$ (mean baseline temp)</td>
<td>35.62</td>
<td>30</td>
<td>.1337</td>
<td>266.39*</td>
</tr>
<tr>
<td>$\beta_{01}$ (effect of skinfold mean)</td>
<td>.0504</td>
<td>30</td>
<td>.0237</td>
<td>2.127*</td>
</tr>
<tr>
<td>$\beta_{10}$ (mean growth rate)</td>
<td>.0072</td>
<td>30</td>
<td>.0004</td>
<td>17.33*</td>
</tr>
<tr>
<td>$B_{11}$ (effect of growth rate)</td>
<td>-.0003</td>
<td>30</td>
<td>.0001</td>
<td>-2.69*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random Effect</th>
<th>Variance Component</th>
<th>df</th>
<th>Std Dev</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{00}$ (error baseline)</td>
<td>.6105</td>
<td>30</td>
<td>.7813</td>
<td>350169.17*</td>
</tr>
<tr>
<td>$r_{1i}$ (error growth)</td>
<td>.000001</td>
<td>30</td>
<td>.0024</td>
<td>139523.83*</td>
</tr>
<tr>
<td>$e_{ii}$</td>
<td>.0063</td>
<td></td>
<td>.0797</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at $p < .05$.

*Mixed Model Repeated Measures for Relative Temperature and Skinfold Thickness*

A non-centered repeated measures model for the response variable relative temperature (°C) regressed onto skinfold thickness (mm) for the treatment period was analyzed. Results demonstrate that skinfold thickness was not predictive of relative temperature for the treatment period ($p = 0.512$) after modeling the repeated effect time with the Compound-Symmetric variance-covariance matrix (Table 19). The prediction equation produced is $Y = \beta_0 + \beta_1 x_1 + \varepsilon$, where $Y$ is the response variable, $\beta_0$ is the
intercept, $\beta_1 = \text{coefficient for skinfold}$, $x_1 = \text{predictor variable measured in millimeters}$, and $\varepsilon = \text{error term}$:

$$\text{Relative temperature} = 37.14 + .024 \times (\text{skinfold thickness}) + \varepsilon$$

Table 19

**MMRM for the Predictor of Skinfold Thickness on Relative Treatment Temperature**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Std Err</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>CI Lower</th>
<th>CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>37.14</td>
<td>.59</td>
<td>30.4</td>
<td>62.19</td>
<td>&lt;.001</td>
<td>35.92</td>
<td>38.36</td>
</tr>
<tr>
<td>MSkinfold</td>
<td>.024</td>
<td>.036</td>
<td>30</td>
<td>.66</td>
<td>.51</td>
<td>-.04</td>
<td>.09</td>
</tr>
</tbody>
</table>

*Note. MSkinfold = mean skinfold thickness.*

Further investigation of this model revealed that the residuals were non-normal (Shapiro-Wilk $p < 0.001$). As a result, the above model was repeated with a log$_e$ and rank transformation of the response variable. Both of these models confirm skinfold thickness to not be predictive of relative temperature (log$_e$: 0.506; rank: 0.531).

**Results Summary**

RQ1: What are the absolute and relative intramuscular and skin temperatures and overall rate of heating per minute for each, respectively, during 3 MHz ultrasound treatment?

Absolute treatment site ended with a mean temperature of 38.2°C, while the relative treatment site ended with a mean temperature of 37.6°C over the treatment period. The skin site ended with a mean temperature of 37.3°C over the treatment period.
Heating rates per each degree above baseline varied during the treatment. The heating rate for absolute went from .84°C to .61°C per minute. For relative, the heating rate also decreased, from .51°C to 42°C per minute, during the treatment period. The skin heating rate decreased from 3.57°C to 2.21°C per minute.

RQ2: Does skin temperature predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?

The OLS regression revealed that skin temperature was not a predictor for absolute temperature during the ultrasound treatment. HLM analysis showed skin temperature to be a significant predictor for absolute temperature during the ultrasound treatment. MMRM analysis revealed skin temperature not to be predictive for absolute temperature during the ultrasound treatment.

The OLS regression revealed that skin temperature was a significant predictor for relative temperature during an ultrasound treatment. HLM revealed that skin temperature was a significant predictor for relative temperature during the ultrasound treatment. MMRM analysis also revealed that skin temperature was a significant predictor for relative temperature.

RQ3: Does subcutaneous skinfold thickness predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?

The OLS regression analysis revealed the inclusion of skinfold thickness was not a significant predictor for absolute temperature during the ultrasound treatment. HLM and MMRM analyses also revealed that skinfold thickness was not a predictor for absolute temperature during the ultrasound treatment.
The OLS regression analysis revealed the inclusion of skinfold thickness was not a significant predictor for relative temperature during the ultrasound treatment. HLM analysis revealed that the inclusion of skinfold thickness was a significant predictor for relative temperature during the ultrasound treatment. MMRM analyses revealed that skinfold thickness was not a predictor for relative temperature during the ultrasound treatment.
CHAPTER V

DISCUSSION

The specific research questions addressed in this study, based upon the purposes are:

RQ1: What are the absolute and relative intramuscular and skin temperatures and overall rate of heating per minute for each, respectively, during 3 MHz ultrasound treatment?

RQ2: Does skin temperature predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?

RQ3: Does subcutaneous skinfold thickness predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?

RQ4: Do the three analysis models (OLS regression, HLM, or MMRM) produce similar results for the research questions?

In order to answer the research questions, an ultrasound treatment was given to subjects under the following parameters: settings of 3 MHz at 1.0 W/cm², using a 2 ERA treatment site, and raising the absolute intramuscular temperature to 3°C above baseline. Skinfold thickness and skin temperature, along with absolute and relative intramuscular temperatures, were recorded.
This discussion begins by answering RQ4 to summarize which analytical technique is more appropriate before addressing RQs 2 and 3. The decision of the appropriate model is then used to interpret RQs 2 and 3 for the discussion section.

RQ4: Do the three analysis models (OLS regression, HLM, or MMRM) produce similar outcomes for the research questions?

Each analytical technique has properties making it suitable for the analysis. However, the best technique to analyze the data should be based upon the research design and individual responses to the treatment. The data collected in this study represent a longitudinal design, with time points repeated in half-second intervals throughout the ultrasound treatment period. These type of data are useful for examining trends (in this case, temperature). As such, several different analysis techniques were employed to examine the relationship of the independent variables (skin temperature and skinfold thickness) on the overall degree change for the ultrasound treatment period.

Because of the methods of data collection, there were no missing temperature points throughout the treatment period for each subject. However, the time it took to raise the temperature to the overall 3°C change varied by person, resulting in different ending points for all subjects. The initial starting temperatures also varied by person. OLS regression used in this study was analyzed based upon the mean ending time for the subjects. Therefore, OLS regression cannot effectively measure individual growth rates or how these growth rates differ within the person during the treatment.

The nonsignificant results may also be based upon the normality of the data. In repeated measures designs, data points measured close together tend to be more
associated than data points collected further in time. The relationship of the data values with a repeated measures study could affect the estimation techniques used in OLS, since OLS uses an end time point for analysis. Because time was included in the model and skin temperature was also a function of time, the two variables should be highly correlated but were found to be correlated only for relative temperature. The high correlations for the relative treatment site can be contributed to the needle depth, which accounted for the adipose thickness of the subjects. When high correlations are present, the coefficients of the model will most likely be significant, which was evident in the results. Additionally, the analyses were checked for violation of assumptions. There were no apparent outliers in the data and multicollinearity using Tolerance and VIF procedures. Thus, the use of the regression analysis was deemed appropriate.

The curvilinear trend for time to achieve the 3°C change over the treatment period may also have accounted for the nonsignificant results. To examine linearity, the data were analyzed using HLM for a quadratic growth versus linear growth. A quadratic growth parameter with the inclusion of skinfold thickness as a level 2 predictor with skin temperature and treatment time as level 1 predictor was examined to determine if the data are curvilinear or linear for absolute and relative temperature sites. Although the quadratic growth parameters were significant, the baseline temperature coefficient changes were extremely small (−0.000007) for absolute and relative (.000005); thus, it can be concluded that the simpler linear model was appropriate since the small values of the quadratic coefficient were almost meaningless from a clinical perspective because the treatment had a narrow temperature restriction. If the temperature ranges were widened,
the linear model may not be appropriate and future studies needed to investigate this phenomenon.

HLM, unlike OLS regression, is designed to examine longitudinal data and trends associated with different levels of the analysis at the individual level, then to examine how these trends are affected by the individual characteristics (Wu, 1996). HLM examines the changes in the trends of the individual, then examines the variations between individuals in the parameter estimates since individuals’ changes differ across subjects (Wu, 1996), which OLS regression cannot. HLM therefore can examine each subject’s trends, the overall group’s trend or trajectory, and the variance of the individual around the mean of the trend, and predict the individual’s differences in the trends (Hernandez-Lloreda et al., 2003). The strength of HLM to develop a growth curve in the model and determine how the independent variables would affect the growth curve was a main reason to use HLM analysis technique. Upon reviewing the results, skin temperature was found to be predictive for both absolute and relative temperatures and skinfold thickness was a predictor for relative temperature. OLS regression could not detect these parameters in the model, most likely due to examination of the model based upon the ending time for the treatment period versus the growth rates of the variables across persons.

MMRM analysis can also predict individual and group growth curves like HLM discussed in Chapter II. The main difference between these two models is how the data were analyzed over time. MMRM analysis divided the treatment time into deciles (10 equal time points), essentially compressing subjects’ time points into equal components, regardless of the differences in the ending time between subjects. The deciles restriction
in MMRM was used to construct the growth patterns, whereas for HLM, the time points were not restricted. The MMRM divided time points for the treatment session may not necessarily be the best technique for the data analysis because it confines the individual growth rates into these time periods for data analysis. Because of possible data restrictions, three methods for violations of normality were conducted for the respective MMRM models. In most cases, normality of the data was violated, likely contributing to the restricted time points and heteroscedasticity of the data.

Upon reviewing the results, MMRM revealed that skin temperature was a predictor for relative temperature similar to HLM; however, HLM found skinfold thickness to be a predictor for relative temperature. Thus, MMRM found only RQ2 to be a predictor for ultrasound treatment.

With measurement of individual and group growth rates versus an analysis based upon an ending temperature only, and without time restrictions utilized in MMRM, the results from the HLM analysis were used as the interpretation for the study. However, contributions from OLS regression and MMRM techniques were used to help strengthen the outcomes in the discussion.

RQ1: What are the absolute and relative intramuscular and skin temperatures and overall rate of heating per minute for each, respectively, during 3 MHz ultrasound treatment?

The heating rate for the total treatment period as a function of time was .61°C at the 1.5 cm depth. This translates to a .010°C per second temperature increase during the entire 3 degree increase. According to Draper, Castel, and Castel (1995), using the same ultrasound parameters of 3 MHz at 1.0 W/cm², the heating rate was .58°C per minute.
Holcomb and Joyce (2003) and Johns et al. (2007a) inserted a temperature probe at 1.2 cm depth and found that the heating rate was also .58°C per minute. In Miller et al., (2008), using 3 MHz at 1.0 W/cm², the heating rate for the ultrasound treatment was .58°C per minute. Hayes et al. (2004) found the heating rates to be 1.19°C per minute when applying ultrasound at 3 MHz at a depth of 2.5 cm using a Rich-Mar ultrasound machine. Additionally, Chan et al. (1998) measured temperature increase in the patella tendon and found a 2.1°C increase in rate per minute using 3 MHZ at 1.0 W/cm².

The depths for which temperature was measured varied slightly. In Draper, Castel, and Castel (1995), the depth was 1.6 cm; Holcomb and Joyce (2003) had a depth of 1.2 cm, and Miller et al. (2008) had a depth of 1.0 cm. Our study had a slightly higher rate per minute (.61°C), which may have been due to fluctuations of subcutaneous tissue thickness, not the depth range. While this study specifically measured subcutaneous thickness during treatment, only one study utilized this variable with ultrasound treatment but used a 1 MHz treatment instead of a 3 MHz treatment (Draper & Sunderland, 1993). Their results showed that subcutaneous thickness was not a factor in heating of the tissues, but the 1 MHz frequency, which is absorbed deeper into tissues, may account for these differences in temperatures.

The temperature rates and overall heating of the intramuscular tissues may also be influenced by the type of ultrasound unit or even the transducer that delivers the ultrasound energy. Holcomb and Joyce (2003) showed that the Omnisound unit had significantly greater increases in temperature and higher heating rates (.58 and .39°C, respectively) compared to the Forte 400 Combo unit. Similarly, Merrick, Bernard, et al. (2003) found that the Omnisound produced greater heating rates versus the Dynatron or
Excel ultrasound units. When examining the effects of different transducers, Demchak et al. (2007) found that although different transducers from the same manufacturer produce curvilinear heating rates during treatment, the overall peak heating after a 10-minute treatment was still within .1°C. Johns et al. (2007b) showed that the ERA over the transducer varies versus the actual claim of the manufacturer, except the Omnisound. These results suggest that when utilizing an ultrasound unit for treatment outcomes, the specific manufacturer and even the transducer may account for slight discrepancies in heating and heating rates.

The Omnisound ultrasound unit also has set temperature parameters for 1, 2, and 4°C, based upon work developed by Draper, Castel, and Castel (1995). When these temperature settings are selected on the machine, the unit calculates the time in which these temperatures should be achieved. Based upon the set time points, at 1°C, the designed time would be 100 seconds; at 2°C, the time would be 206 seconds; at 3°C, the time would be 310 seconds. However, this study had heating rates quicker at the 1°C time (71 seconds), 2°C time (167 seconds), and 3°C (294 seconds) time frames.

The role of skinfold thickness may have contributed to the time to achieve critical temperatures. With relatively lean skinfold thickness of the subjects, the intramuscular tissue may have heated more quickly, especially in deeper intramuscular tissues. Since the heating rates of the aforementioned articles do not discuss the effects of skinfold thickness, this variable could be an influencing phenomenon for ultrasound treatments.

The heating rates per minute and overall intramuscular heating temperature may also be a function of the methodology used to determine temperature outcomes. In Chan et al. (1998), treatment lasted for 4 minutes, regardless of overall temperature. In
Holcomb and Joyce (2003), the temperature was elevated to 6°C above baseline or 10 minutes, whichever came first. Draper, Castel, and Castel (1995) and Miller et al. (2008) provided treatments for 10 minutes or until the subjects complained of discomfort. Similarly, Hayes et al. (2004) discontinued treatment either after 10 minutes or until a critical temperature of 40°C was found over consecutive temperature readings of a minute. With these variations in methodology, heating rates may be difficult to estimate uniformly for ultrasound treatments. This study reported heating rates per degree change; however, in the aforementioned studies, their rates were based upon ending treatment temperature per unit of total time. Since ultrasound heating appears to be curvilinear, heating rates should be faster initially, then level off or decrease over time, similar to the results from this study. But based upon a 3°C temperature change limitation, a true curvilinear response may not be present when narrowing the temperature ranges to the treatment period only.

The skin and relative sites also had similar trends in heating rates. As shown in Table 5, when the absolute temperature reached 1°C above baseline, skin temperature averaged 30.61°C with a heating rate of 3.57°C; at 2°C above baseline, the heating rate was 2.80°C; and at 3°C above baseline, the heating rate was 2.21°C. Relative heating rates also decreased over the treatment period but at a slower rate. At 1°C above baseline, the rate was .51°C; at 2°C above baseline, the rate was .47°C; and at 3°C above baseline, the rate was .42°C. It may be postulated that the depth of the relative site may account for the lower heating rate per time. In addition, the relative temperature site did take into account the amount of subcutaneous tissue for each subject, which theoretically means the same amount of muscle tissue was exposed during treatment, while the absolute site
had varying muscle tissue exposure based upon skinfold thickness. The amount of temperature held within muscle or subcutaneous tissue may account for these differences and should be explored.

The dissertation results warrant further investigation into these heating rate fluctuations. As can be seen in Table 5, the heating rates appear to be curvilinear and calculations based upon a specific ending temperature and total time do not effectively describe the rate of change over the entire ultrasound treatment. The published ultrasound studies that reported heating rates and temperature changes always use the ending temperature to predict and regress backwards for their suggested heating parameters. Based upon the results of this study, it may be prudent to develop heating rates for each .5°C change.

RQ2: Does skin temperature predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?

Skin temperature was a significant predictor for the absolute temperature when modeled with HLM analysis. For the relative depth, skin temperature was also a significant predictor in the HLM and MMRM models. As seen in Figures 4–6 and Tables 4 and 5, temperatures increased steadily during the treatment. With skin temperature as a significant predictor for absolute and relative depths, the use of the model equations developed with the HLM analysis can now be used for determining intramuscular temperature interpretations versus actually measuring intramuscular temperature with thermocouples. The formula

$$Y_t = \beta_{00} + \beta_{10} \text{ (skin)} + \beta_{20} \text{ (time)} + r_{01} + r_{11} \text{ (skin)} + r_{21} \text{ (time)}$$

...
+ $e_i$ can be used for both absolute and relative intramuscular depths when using skin temperature for predicting intramuscular temperatures.

These results provide evidence that skin temperature during ultrasound treatments can be used to predict intramuscular heating; however, it appears that the relationship is stronger for the relative depth, where the distance from the surface of the treatment site is greater and where the amount of muscle tissue exposed is relatively the same across participants. In this study, the maximum depth was 28 mm and the average depths were within the ranges suggested for the use of 3 MHz according to Hayes et al. (2004) and Draper and Prentice (2003). This dissertation used a 3 MHz ultrasound frequency, which can target tissue depth up to approximately 25 mm and further investigation is warranted to examine the influence of skin temperature using greater depths with 1MHz frequency ultrasound treatments.

Using skin temperature as a predictor during cryotherapy showed similar results with this study. Jutte et al. (2001) found that skin temperature predicted 21% of the variance and may have been influenced by several variables, including temperature increases after removal of the modality, area exposed, and duration of cooling. While Jutte et al. examined cooling rates with cryotherapy, they postulated that other factors, such as room temperature, adipose thickness, and core temperature in combination may better explain intramuscular temperatures. Similar to factors of variability in Jutte et al., room and gel temperature may also have contributed to the outcome of the present study. However, gel temperature was measured over several occasions and was found to be within .5°C degrees during the data collection period. Room temperature was not
measured during the data collection period and may have been a confounding variable that should be controlled for future studies.

RQ 3: Does subcutaneous skinfold thickness predict absolute and relative intramuscular temperatures during 3 MHz ultrasound application?

Subcutaneous tissue thickness has been shown to alter heating and cooling of muscle (Johnson et al., 1979; Jutte et al., 2001; Lowdon & Moore, 1975; Myrer, Myrer, et al., 2001; Otte et al., 2002; Zemke, Anderson, Guion, McMillan, & Joyner, 1998), but is confined to the use of cryotherapy where adipose acts like an insulator. After the underlying tissues are cooled, adipose tissue slows the rate of re-warming by keeping the tissues cool and plays a significant role in intramuscular temperature rises and decays over time. According to Myrer, Myrer, et al. (2001), the adipose thickness plays a significant inverse relationship to the rate of cooling. Subjects with an 8 mm thickness or lower had faster rates of cooling compared to subjects with 20 mm or greater adipose thickness. Adipose thickness and cooling rates showed that the thicker the adipose layer, the longer the cooling time (Otte et al., 2002). However, past research has also shown that adipose thickness was weakly related to intramuscular temperature, accounting for 14% of the variance during cryotherapy treatment and was a weak predictor for intramuscular temperature (Jutte et al., 2001).

Draper and Sunderland (1993) measured muscle temperature 3 cm below the surface of the calf muscle, and then subtracted the subcutaneous thickness to determine how adipose thickness affects heating. Subjects then were divided into two groups, more than 10 mm or less than 10 mm adipose thickness. Results showed that both groups had
similar heating temperatures 4.9 ± 1.0 to 4.8 ± 1.7°C. In their study, however, a total time of 10 minutes or until three consecutive temperature readings were recorded determine the treatment time. In addition, they did find a small nonsignificant positive correlation between adipose and tissue temperature, which could be a result of their methods.

The absolute treatment site, for this current study, was 1.5 cm for all subjects but the depth within the muscle tissue varied according to the thickness of the adipose. With a range from 8 to 26 mm, temperature was measured from 7 to 1.1 mm of muscle depth. The depth variation in the muscle tissue could explain the nonsignificant findings, which can be supported by our low percentage of variability explained in the results when adipose was added into the model for HLM, accounting for only 4% more of the variability.

To account for adipose thickness and to ensure that all subjects had the exact same thickness of muscle tissue targeted, the relative depth in this study was calculated as 1.5 cm plus half the thickness of the skinfold measurement. The relative depth took into account the adipose thickness and all subjects then had similar muscle tissue thickness treatment with ultrasound energy. For example, for a subject with 8 mm adipose thickness, the relative depth was 1.5 cm plus 4 mm, which resulted in a depth of 1.9 cm or a muscle thickness of 1.5 cm. For a subject with 16 mm adipose thickness, the depth was calculated as 1.5 cm plus 8 mm or 2.3 cm depth, resulting in a muscle thickness of 1.5 cm. Hence, all the subjects for the relative depth had the same amount of muscle tissue exposed during the treatment, regardless of the amount of adipose overlying the muscle.
Using HLM, the results showed that skinfold thickness was a predictor for relative
temperature and also showed that an inverse relationship existed with the growth rate.
Again, the model developed based upon the HLM results can be used by clinicians to
predict intramuscular temperature based upon skinfold thickness, but only at the relative
site. The formula is as follows: \( Y_u = \beta_{00} + \beta_{01}(\text{skinfold}) + \beta_{10} (\text{skin temp}) + \beta_{11} (\text{skin temp} \times \text{skinfold}) + \beta_{20} (\text{time}) + \beta_{21} (\text{time} \times \text{skinfold}) + r_{01} + r_{11} (\text{skin temp}) + r_{21} (\text{time}) + e_u \)

These results were not supported by Draper and Sunderland’s (1993) findings.
The differences between this dissertation results and Draper and Sunderland could be
explained by two specific factors. As mentioned previously, the leanness of the subjects
at the targeted treatment area could account for the findings. To determine if adipose does
have a higher contribution to the overall heating effects for the absolute depth,
recruitment of subjects with a wider range of adipose thickness should be conducted.
Adipose thicknesses should be divided into ranges based upon millimeter thickness,
either in two or three groups, and then comparisons should be made.

The second factor that may explain the different findings with skinfold thickness
could be the energy absorption of adipose with the respective ultrasound frequency.
Castel et al. (1998) found that using a 3 MHz ultrasound frequency at 1.0 W/cm\(^2\) for 10
minutes, the adipose layer can be heated vigorously, peaking at a mean temperature of
41.4°C or 8.4°C above baseline. These results provided evidence that adipose tissue does
absorb ultrasound energy to some extent, possibly more than previously thought.

As can be seen with the results from this study for the relative depth, the role of
adipose may be a contributor to ultrasound heating. These results may also show some
evidence for adipose thickness variations, with individuals with higher adipose thickness
absorbing more energy than those with minimal thickness. Draper and Sunderland (1993) used a 1 MHz frequency in their study and determined that adipose thickness did not influence heating of the underlying tissues, while this dissertation used 3 MHz. Future studies need to specifically examine a wider range of adipose thickness, ultrasound frequency, and treatment time, and combinations of these variables to determine the influence for heating intramuscular tissues.

Summary and Conclusions

Although there were no missing data points, the length of time to reach the critical temperature varied within subjects and the use of an ending time point to predict temperature with OLS regression may not be appropriate. HLM, on the other hand, develops a growth rate from the initial treatment temperature and then determines how the independent variables affect the growth. These results allow for individual fluctuations, whereas OLS regression cannot. With MMRM, restrictions to the time points may have contributed to the nonsignificant results; however, this model, similar to HLM, is more appropriate than OLS regression. OLS regression, which utilizes mean values of ending time points, cannot explain how individual growth rates may account for predictor variables.

At the end of the treatment, the absolute site had a mean temperature of 38.2°C, while relative temperature site ended with a mean temperature of 37.6°C. Skin temperature ended with a mean temperature of 37.3°C. Heating rates per each specific degree above baseline varied during the treatment. The heating rates for absolute went from .84°C to .61°C per minute. For relative, the heating rates also decreased, from .51°C
to .42°C per minute during the treatment period. Finally, the skin heating rates decreased in similar fashion, from 3.57°C to 2.21°C per minute. The overall rate per minute of the entire treatment time for absolute was .61°C at the 1.5 cm depth, which is slightly higher than other studies in which the rate with similar ultrasound settings was .58°C.

HLM showed that skin temperature is a significant predictor of absolute and relative temperatures, while MMRM found only skin temperature to be a predictor of relative depths during the ultrasound treatment. OLS did not find significance with skin temperature on both absolute and relative temperatures. The HLM model developed can be used by clinicians for estimating heating effects for their patients. The significant skin temperature prediction for relative depth may be based upon the uniformity of the amount of muscle tissue exposed when subcutaneous tissue is taken into account.

OLS regression and MMRM analytical models demonstrated that skinfold thickness is not a predictor for absolute and relative temperature during the treatment period. HLM did find skinfold thickness to be a predictor for relative temperature. However, the varying skinfold thickness may have prevented strong conclusions as to the extent of heating or energy absorption with 3 MHz ultrasound treatments for the absolute depth.

Recommendations (Ultrasound Research)

1. To determine the extent of subcutaneous thickness (skinfold thickness), a large sample of skinfold ranges should be tested. Subjects should be divided into four groups based upon skinfold thickness, ranging from 0–11 mm, 11–20 mm, 21–30 mm, and 31–40 mm. These values are based upon previous experience with skinfold thicknesses and
electrical stimulation and these recommended values are needed to further expand how skinfold thickness may affect heating at smaller depths associated with the absolute depth site in the current study.

2. More research should be conducted over a larger sample to explore how heating rates vary by time and degree reached within the tissues. Heating rates by degree are curvilinear and heating rates should be developed based upon segmented temperature and time components.

3. The use of skin temperature appears to be a method for determining intramuscular temperature, but only for depths used in this study and for 3 MHZ ultrasound treatments. More research is needed to find the effects of skin temperature with different ultrasound frequencies and for varying tissue depths.

4. This dissertation investigated only temperature changes during the treatment period without examining recovery temperatures. The recovery temperature and time to return to initial treatment temperature values may impact the clinical ramifications between the absolute and relative depths, especially in patients with more subcutaneous tissue thickness; therefore, future research should explore these variables.

Recommendations (Statistical)

1. The use of longitudinal data, such as temperature during ultrasound treatments, should be analyzed by HLM. HLM appears to be more appropriate for the analysis since the procedure accounts for individual differences and group differences, while OLS regression is based upon mean group data. MMRM, similar to HLM, can be another method to analyze repeated measures, but imposing equated time frames in the current
study may have confounded the results. Thus, more research is warranted to compare HLM and MMRM techniques.

2. During the writing of this dissertation, very few published manuscripts reported or discussed the use of analytical models for their data. As such, the use of certain statistical models may not be suited for the type of data collected. Violations of normality must be conducted and reported for all published reports so that future researchers can determine the most appropriate analysis methods.

3. The use of HLM and MMRM for analyzing ultrasound research or other research involving temperature or time data should be explored since these analytical techniques have been predominantly used in educational research.

4. A more comprehensive investigation should be explored to examine the curvilinear characteristics (both treatment and recovery periods) without the 3°C ultrasound heating limitation of this dissertation.
REFERENCES


Appendix A

Power Analysis
Power Analysis Criteria using Multiple Regression Model

### Analysis:
A priori (Multiple Regression: Omnibus ($R^2$ deviation from zero)

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<tr>
<td>Power ($1-\beta$ err prob)</td>
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Appendix B

Recruitment Flyer
You are invited to participate in a research study concerning intramuscular temperature and therapeutic ultrasound.

**PURPOSE:**
This study will investigate changes muscle tissue temperature as a result of a therapeutic ultrasound treatment. This information will help clinicians and rehabilitation professionals better understand the effects, benefits, and protocols of therapeutic ultrasound.

**WHO:**
Healthy individuals between the ages of 18-50. Potential applicants will be free of any lower extremity injury within the last 6 months. They will also be free of any vascular or nervous condition which may alter blood flow or temperature sensations. Applicants should also not currently be pregnant.

**VISITS:**
The research treatment will be completed in one visit. The session will include a consent to participate, a health questionnaire, as well as an explanation of the benefits and risks of participation in the study. If you elect to participate, a skin fold measurement will be taken over the calf, followed by the insertion of 2 intramuscular temperature needles. These needles will remain in the muscle for the duration of the study. Then you will be given an ultrasound treatment (approx. 10 minutes). The whole process should last approximately 90 minutes. Since you will be asked to lie on your stomach and relatively motionless for approximately 90 minutes, we suggest you bring homework, readings, etc.

**WHERE:**
Western Michigan University
Student Recreation Center (1st floor)
Human Performance Research laboratory

If you have any questions or would like to learn more about participating, contact:

Dr. Michael G. Miller (269) 387-2728
-OR-
michael.g.miller@wmich.edu
Appendix C

Human Subjects Institutional Review Board
Letter of Approval
Date: September 23, 2009

To: Brooks Applegate, Principal Investigator  
    Christopher Cheatham, Co-Principal Investigator  
    Michael Miller, Student Investigator for dissertation

From: Amy Naugle, Ph.D., Chair

Re: HSIRB Project Number: 09-08-03

This letter will serve as confirmation that your research project titled "Absolute and Relative Temperature Changes during Ultrasound Treatment" has been approved under the full category of review by the Human Subjects Institutional Review Board. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

Please note that you may only conduct this research exactly in the form it was approved. You must seek specific board approval for any changes in this project. You must also seek reapproval if the project extends beyond the termination date noted below. In addition if there are any unanticipated adverse reactions or unanticipated events associated with the conduct of this research, you should immediately suspend the project and contact the Chair of the HSIRB for consultation.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: September 16, 2010
You have been invited to participate in a research project titled "Absolute and Relative Tissue Temperature Changes During Ultrasound Treatment." This project will serve as a research and dissertation project for the requirements of the Evaluation, Measurement, and Research PhD degree for the Michael G. Miller. This consent document will explain the purpose of this research project and will go over all of the time commitments, the procedures used in the study, and the risks and benefits of participating in this research project. Please read this consent form carefully and completely and please ask any questions if you need more clarification.

What are we trying to find out in this study?
The goal of this study is to find out how effective ultrasound treatment is as heating the muscle at different depths. A second goal is to improve the ability of predicting how long ultrasound treatment needs to last in order to increase muscle temperature to a given level.

Who can participate in this study?
If you are a male or female, within the age range of 18-45 years, and not have recent leg injuries (within the past 6 months), infections, vascular or nervous conditions, a pacemaker, or pregnant (female subjects only) you can participate. If you meet these criteria and agree to participate, you will be given a brief medical questionnaire. If any item in the medical questionnaire results in a physical condition not to participate, you will be removed from the study.

Where will this study take place?
The study will take place in the Human Performance Research Laboratory (HPRL) in the first floor of the Student Recreation Center at Western Michigan University.

What is the time commitment for participating in this study?
As a subject, you will report to the HPRL at a designated time for approximately 90 minutes. Approximately 30 minutes will be required for prepping you for the procedure, 20 minutes for baseline measures, 10 minutes for treatment and 20 minutes for recovery and 10 minutes for removal of probes and re-cleaning the area on your leg.
What will you be asked to do if you choose to participate in this study?
You will lay on your stomach on a treatment table for comfort and accessibility. The skin on your calf will be shaved and thoroughly cleansed using a Betadine scrub followed by isopropyl alcohol pads over the mid-point of the calf muscle. A skinfold measure will be taken using calipers. Three temperature probes will be utilized for the study. One probe will be placed on the skin surface and two will be placed into the calf muscle after application of a cold spray to numb the area. One probe will be inserted 1.6 centimeters below the top of the calf and the other probe will be inserted approximately 2-4 cm below the top of the calf. The probes are about the thickness of a flu shot needle but about 1-2 cm longer. The researcher is trained in the application of these probes by a physician and has conducted several studies utilizing this technique. Once the probes are applied or inserted into your calf, baseline temperature will be recorded for 20 minutes. The ultrasound heating treatment will last for 10 minutes or until muscle temperature increases 3°C from baseline at the closest probe depth. The ultrasound treatment consists of moving a ultrasound head over the top of your calf in the designated area. You should experience slight warmth during the application. Following the end of the treatment the ultrasound unit will be turned off and temperature will be recorded for 20 more minutes. Upon completion, your calf will be re-cleansed using isopropyl alcohol pads. You will be instructed on the use of an ice bag to prevent soreness over the treatment area even though the use of the cold spray applied prior to the insertion of the probes should diminish any soreness. Information for signs and symptoms of possible infection will be given to you in a written format upon completion of the study session. If you experience any discomfort in your calf after participating, you need to contact Michael G. Miller by email or phone.

What information is being measured during the study?
An ultrasound heating unit will be applied to increase temperatures to the calf. Surface and calf muscle temperatures will be recorded using a specialized temperature machine.

What are the risks of participating in this study and how will these risks be minimized?
You will be properly instructed about the protocol before and during treatment, and will be given proper consideration if any discomfort should arise. The ultrasound treatment will heat the calf and treatment and will cease if you report pain, burning or discomfort. The use of ultrasound gel should minimize the heating sensation. Inserting probes into the skin may cause discomfort. Ethyl chloride (cold spray) will be applied to the skin prior to probe insertion to minimize any discomfort. Cleaning the area and providing written instruction on signs and symptoms of infection will be provided for you. You have the right not to participate in this study, and the freedom to withdraw at anytime without prejudice or penalty. Your refusal to participate will not affect your grade or academic status, and if you choose to discontinue participation, you have the right to withdraw your data as well. You will also be given contact information to the Chair, Human Subjects Institutional Review Board or the Vice President for Research if questions or problems arise during the study.
What are the benefits of participating in this study?
The results will give clinicians beneficial information about how certain ultrasound parameters can be used to treat and heat certain injuries. However, these benefits are subjective, and there may be no direct benefit to you. Although not a benefit, subjects who complete the study will be provided a t-shirt.

Are there any costs associated with participating in this study?
There are no anticipated costs for taking part in the study. However, should an infection arise, you may use the Sindecuse Health services at WMU for treatment at your expense.

Is there any compensation for participating in this study?
You will not receive compensation, class credit, extra credit or any other form of incentive for your participation.

Who will have access to the information collected during this study?
An identification number (rather than your name) will be used to record the data. The connection between your name and subject number will be known only to the investigators. Following the study, the primary investigators will have access to the original data. The original data will be retained in a locked storage unit for a minimum of three years after the completion of the study in the department of Health, Physical Education, and Recreation at Western Michigan University then destroyed. Your right to privacy will be protected at all times. The data and results from this study may be present or published in a scientific/medical journal, but your name will not be included in anything.

What if you want to stop participating in this study?
You can choose to stop participating in the study at anytime for any reason. You will not suffer any prejudice or penalty by your decision to stop your participation. You will experience NO consequences either academically or personally if you choose to withdraw from this study. The investigator can also decide to stop your participation in the study without your consent.

Should you have any questions prior to or during the study, you can contact the primary investigator, Dr. Michael G. Miller at 269-387-2728 or michael.g.miller@wmich.edu. You may also contact the Chair, Human Subjects Institutional Review Board at 269-387-8293 or the Vice President for Research at 269-387-8298 if questions arise during the course of the study.

This consent document has been approved for use for one year by the Human Subjects Institutional Review Board (HSIRB) as indicated by the stamped date and signature of the board chair in the upper right corner. Do not participate in this study if the stamped date is older than one year.
I have read this informed consent document. The risks and benefits have been explained to me. I agree to take part in this study.

Please Print Your Name

Participant’s signature               Date
Appendix E

Health History Questionnaire
HEALTH HISTORY QUESTIONNAIRE

Mark "YES" or "NO" to the following questions.

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