School of Physiotherapy

An evidence-based model for determining treatment dosages in therapeutic ultrasound using thermometry: an in-vitro investigation using post-mortem pig tissues

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

Signature: .....

Date: 14 FEB 2003

Statement of Sources

This series of studies was initiated following review of the relevant literature and discussions with Professor Takayuki Fujiwara (Shinshu University, Japan) and Professor Joan Cole (Curtin University of Technology, Australia).

The planning, organization and management of the studies were the sole responsibility of the author. The development of the experimental design, applications for funding and the presentation of the results, were undertaken by the author in consultation with thesis supervisors Professor Joan Cole, Professor Takayuki Fujiwara and Dr Kathy Briffa.

Statistical analyses and interpretation of the data were the sole responsibility of the author. Advice and guidance in these matters were sought and obtained from Dr Marie Blackmore, from the School of Physiotherapy, Curtin University of Technology.

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I hereby declare that the work presented in this thesis, is to the best of my knowledge and belief, original, except as acknowledged in the text and that the material, either whole or in part, has not been submitted for a degree at this or any other University.

Gon An Cheng

Abstract

The aim of this study was to clarify the relationship between the dosage parameters and temperature increase at the target tissues (up to 5 cm below the skin surface), and to explore the possibility of proposing a preliminary model to guide clinicians and researchers in determining treatment dosages based on expected increase in temperatures at the target tissue. Prior to the conduct of the main study several protocol-related issues were investigated. These included the reliability of the measurement procedures, the optimum speed of movement of the transducer, the optimum size of the treatment area, and the maximum output intensity that could be considered safe for treatment applications and investigations.

An in-vitro post-mortem pig model was chosen for the experimental design using only adult-sized pigs, weighing between 60 to 80 kilograms. A total of 76 specimens were obtained from the shoulder and thigh sections of 19 pigs.

The therapeutic ultrasound machine used throughout the study was the Omnisound 3000™ (Physio Technology Inc., Topeka, Kansas, USA). Output from the Omnisound 3000™ was checked and calibrated as necessary prior to each experiment using a power meter (Model UPM-DT-10, Ohmnic Instruments Co., St. Michaels, Maryland 21663, USA). Calibration was only performed when the checks demonstrated an error in the output intensity of the machine exceeded ±10%. The Minolta spot thermometer (HT-11, Minolta Co. Ltd., Japan) and the Avio thermal video system (TVS) 2000™ (Nippon Avionics Co. Ltd., Japan) were used to measure the change in tissue temperatures (dependent variable) at the skin surface and subcutaneously (at 1, 2, 3, 4 and 5 cm below skin surface) respectively.

The prepared specimen was mounted on a fixed table, with the clean

cross-section facing the infrared thermographic camera. The camera to specimen distance was standardised at 50 cm for all experiments. Markers corresponding to 1, 2, 3, 4, and 5 cm on the specimen were plotted on the display unit, and saved to a 3.5 inch floppy disk. Measurements were recorded at baseline (prior to commencement of the experiment) and subsequently at 1-minute intervals during 10 minutes of exposure to the ultrasound, and for a further 10 minutes post-exposure, until the end of the experiment at 20 minutes. In general, there were five main parameters for all the studies: the movement speed of the transducer, the size of the treatment, and the frequency, intensity and duration of exposure and post-exposure to ultrasound. These five parameters represented the independent variables for all the studies. The dependent variable throughout was change in tissue temperature (measured in °C) at the skin surface, and at 1, 2, 3, 4 and 5 cm below the skin surface.

Data were analysed using the SPSS for Windows software, Version 10.0 (SPSS Inc., 444N Michigan Avenue, Chicago, Illinois 60611, USA). Analyses of the data, using a repeated measures analysis of variance procedure, were performed on change in temperature, rather than actual temperature measured at selected time points. Only data from the 5^{th}, 10^{th}, 15^{th}, and 20^{th} minutes were analysed. This corresponded to the middle and end of the ultrasound exposure phase (5^{th} and 10^{th} minute) and post-exposure phase (15^{th} and 20^{th} minute), as these were considered to be representative of both these phases of data collection. Data for all 20-minute sampling is provided in the table of means for each experiment. The level of statistical significance was set at 0.05.

Results of the reliability study showed that both the infrared spot thermometer and the video thermography unit were reliable within acceptable limits (as defined in this study). The latter, however, was more reliable than the former. In addition, the reliability was better for the post-exposure phase compared with the

exposure phase, and for deeper tissues compared with the superficial and surface tissues. An unplanned analysis of the twenty minutes of data (at one minute intervals) suggested the possibility of reducing the duration factor from 20 to 4 (5^{th}, 10^{th}, 15^{th} and 20^{th} minute). In this manner, the data analyses for subsequent studies could be simplified considerably without affecting the overall results.

Results of the other protocol-related studies showed that:

a. There was no difference in change in temperatures between the slow (60 beats/min or 7cm/s), moderate (120 beats/min or 14cm/s) and fast (180 beats/min or 21cm/s) movement speeds of the transducer. However, for practical reasons, the moderate speed was recommended for subsequent studies.

b. There was a significant difference in change in temperatures between the small (2X ERA), medium (3X ERA) and large (4X ERA) treatment sizes. The small treatment size provided the most effective and deeper heating, and was the recommended treatment size for subsequent studies.

c. For both 1 and 3 MHz, tissue damage did not occur for intensities up to 1.5 Watts/cm^2. However, irreversible thermal injury to the tissues occurred at 2.0 Watts/cm^2 (1 MHz). Therefore, the recommended maximum intensity at which investigations could be carried out without any risks of thermal injury to the tissues was 1.5 Watts/cm^2 for both 1 and 3 MHz.

The results from the main study demonstrated that the increase in temperature due to absorption of the ultrasonic energy at any of the investigated target sites (up to 5 cm below surface) was related to the ultrasound frequency, intensity and duration of exposure. For the frequency factor, the evidence seems to suggest that compared with the 3 MHz ultrasound, the 1 MHz frequency may be more appropriate for clinical applications as it does not overheat surface tissues, and at the same time, is

able to increase the temperatures of target tissues up to a depth of 5 cm. For the intensity factor, the results suggest that the therapeutic range of intensities which can be considered neither too low (as to be ineffective) nor too high (as to be damaging) are 0.5 to 1.3 Watts/cm^2 and 0.3 to 0.5 Watts/cm^2 for 1 and 3 MHz respectively. The narrow therapeutic range for 3 MHz could render it questionable for clinical applications. In contrast, the larger therapeutic range available for the 1 MHz frequency suggests that it is more suitable for clinical applications and research. For the duration factor, the results demonstrated that the temperatures at all tissue sites increased as the duration of exposure increased. However, for the post-exposure phase, while the superficial tissues decreased with time, the deeper tissues continued to increase in their temperatures, albeit gradually. In summary, the results demonstrated that a higher frequency, a higher intensity, a greater exposure time and a more superficial site all contribute to a greater change in mean temperature.

From these results, a preliminary model to guide clinicians and researchers in determining treatment dosages, based on expected increase in tissue temperatures at the target site, was proposed. While the preliminary model provided is only a first step effort, it is hoped that it can be refined further through use by physical therapists and other users of therapeutic ultrasound.

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List of Relevant Publications or Presentations

Goh AC, Chok B, Wong WP, Fujiwara T. Therapeutic Ultrasound: Rate of Usage, Knowledge of Use, and Opinions on Dosimetry. Physiotherapy Singapore, 2(2):69-83, 1999.

Goh AC. Electrotherapy and Age Related Changes (Plenary Lecture). 3rd National Congress of the Singapore Physiotherapy Association, 3-4 April, Singapore, 1999.

Goh AC. Towards a realistic model for determining dosages in electrotherapeutic treatment (Workshop). 3rd National Congress of the Singapore Physiotherapy Association, 3-4 April, Singapore, 1999.

Goh AC, Fujiwara T, Cole, J. Relationship between temperature rise and the frequency-intensity of therapeutic ultrasound: An in-vitro investigation using post-mortem pigs. 13th International Congress of the World Confederation of Physical Therapy, 23-28 May, Yokohama, Japan, 1999.

Goh AC, Fujiwara T. The determination of treatment effectiveness in Physical Therapy: In the Area of Electrophysical Agents. Journal of the Japanese Physical Therapy Association, 27(8): 288-297, 2000 (in Japanese).

Goh AC. Electrophysical Agents: Where is the evidence? (Seminar). 35th National Congress of the Japanese Physical Therapy Association, May, Kagoshima, Japan, 2000.

Goh AC, Fujiwara T. Thermotherapy for myogenic pain syndrome. Journal of Physical Therapy (Japan), 18(5): 493-499, 2001 (Japanese).

Goh AC. The use of electrophysical agents by physiotherapists in the new millennium (Plenary Lecture). 1st WCPT-AWP International Congress, 19-23 July, Singapore, 2001.

Goh AC. Recent advances in therapeutic ultrasound dosimetry (Workshop). 1st WCPT-AWP International Congress, 19-23 July, Singapore, 2001.

Goh AC. Evidence based practice in electrophysical agents (Plenary Lecture). 96th Annual Congress of the Nagano Physical Therapy Association, 25th August, Nagano, Japan 2002.

Goh AC. Electrophysical Agents: From evidence to practice (Plenary Lecture). 10th Congress of the Japanese Physical Therapeutics Society, 2-3 November, Kanazawa, Japan 2002.

List of Contents

Page No.

List of Contents (cont.) Page No.

List of Contents (cont.) Page No.

List of Contents (cont.) Page No.

List of Contents (cont.) Page No.

List of Contents (cont.) Page No.

List of Contents (cont.) Page No.

List of Contents (cont.) Page No.

List of Contents (cont.) Page No.

List of Tables

List of Tables (cont.)

List of Tables (cont.)

List of Figures

List of Figures (cont.)

List of Figures (cont.)

List of Figures (cont.)

List of Figures (cont.)

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Ultrasound for medical use has been reported since the end of World War I (Fyfe and Bullock 1985; Nyborg 2001). Since then, the development of ultrasound has led to its usage in medicine, dentistry and physiotherapy. In medicine, ultrasound has been successfully used in the areas of diagnosis (Rumack et al 1998), guided surgery (McGahan 1990), the treatment of cancer cells by hyperthermia using focused ultrasound (Harari et al 1991; Barkman et al 1999), non-invasive surgery such as shock wave lithotripsy for the treatment of kidney stones (Demirkesen et al 2001; Haupt et al 2001) and more recently for ultrasonic liposuction (Grippaudo et al 2000; Cooter et al 2001). In dentistry, ultrasound is used for dental plaque removal (Harpenau 2000; Zimmer et al 2000) and minor dental surgery (Smith 2001). In physiotherapy, therapeutic ultrasound has been used for the treatment of various soft tissues injuries and other pathological conditions (Falconer et al 1990; Feine and Lund 1997; Hay-Smith and Reed 1997; van der Heijden et al 1997).

Ultrasound is a form of mechanical wave motion, similar to that of audible sound, except that it is much higher in frequency (greater than 16 kHz) and inaudible to the human ear (Low and Reed 2000). A mechanical wave motion is defined as "one in which energy is transmitted by the vibrations of the molecules of the medium through which the wave is traveling (gas, solid, or liquid)" (ter Haar 1987, p.110).

The observed and hypothesized biophysical effects of ultrasound are due to this transmission through absorption of the energy within the tissues (Nyborg 2001).

Early laboratory experiments in ultrasound conducted *in vitro* and *in vivo* were motivated both by curiosity about its purported effects and its potential clinical applications, as well as by the need to investigate any adverse effects which could limit its application (Nyborg 2001). Sustained research activities through the decades have led to an increase in theoretical knowledge and better understanding of mechanisms, and this has resulted in improved clinical effectiveness, especially in medicine and dentistry, and to a lesser extent in physiotherapy. However, despite more than six decades of clinical usage and research, there is still only limited understanding of the mechanisms for the observed effects (Nyborg 2001).

During the 1920s and 1930s, attempts were made to learn about the effects of directing ultrasonic energy through biological tissues (Nyborg, 2001). From these early studies, as well as from more recent cellular and animal studies, ultrasound has been shown to have an effect on the inflammatory process (Dyson 1987, Young and Dyson 1990, De Deyne & Kirsch-Volders 1995), on decreasing nerve conduction velocity (Madsen & Gersten 1963, Farmer 1968, Cosentino et al 1983), and in the facilitation of tissue healing (Byl et al 1993, Huang et al 1999).

There have also been many clinical studies in therapeutic ultrasound that have attempted to investigate its effectiveness in various medical conditions. The quality of the evidence from these clinical trials have been assessed in several reviews (Falconer et al 1990; Feine and Lund 1997; Hay-Smith and Reed 1997; van der Heijden et al 1997; Houghton 1999, van der Windt et al 1999; Robertson and Baker 2001) and meta-analyses (Gam and Johannsen 1995; Johannsen et al 1998), based on a randomized controlled trial (RCT) methodological criteria such as the randomization of subjects into experimental and control groups, subject and assessor

blinding, adequate sample size, among others. Some of these reviews concluded that ultrasound was effective (Houghton 1999, Johannsen et al 1998). Others claimed that it was ineffective (Gam and Johannsen 1995, Hay-Smith and Reed 1997, van der Heijden et al 1997, Robertson and Baker 2001), and yet others were unsure or ambivalent (Falconer et al 1990, Feine and Lund 1997, van der Windt et al 1999). Almost all these reviewers were unanimous in criticizing the lack of high quality RCTs for or against the use of therapeutic ultrasound, and that there was an urgent need to conduct better quality studies to resolve this issue. The fact that the evidence from these reviews has been conflicting and ambiguous suggests that much more needs to be understood before the biophysical effects demonstrated *in vitro* and *in vivo* in cell and animal studies can be replicated in human subjects. These issues go beyond mere methodological designs or statistical analyses. Bradnock et al (1995) and Laakso et al (2002) alluded to some of these basic issues that have not been satisfactorily addressed as yet. They suggested that:

- Optimum parameters of treatment dosages including intensity, use of continuous or pulsed mode, ultrasound frequency and duration of treatment have not been established (Bradnock et al 1995, Laakso et al 2002).
- The effects of varying these parameters have also not been investigated (Bradnock et al 1995).
- The effect of inter-patient variation, such as the depth of target tissue and the tissue composition and distribution within the target site, on the dosage parameters is also unknown (Laakso et al 2002).

Bradnock et al (1995) suggested that in the absence of this basic knowledge, the therapeutic norms that have emerged might have no defensible basis as they are largely set by the manufacturers of the equipment rather than by the practitioners (Bradnock et al 1995). It is possible that by following such arbitrary and

questionable guidelines both in clinical practice and research, the true effectiveness of the ultrasonic treatment may be obscured. This may explain, to some extent, the current ambiguity surrounding the effectiveness reported in the various clinical trials conducted to date.

Based on present incomplete knowledge, it would appear to be useful to take a step back from clinical studies, and to re-examine the basic assumptions in terms of their relationship to clinical applications. In particular, the issue of dosimetry must be resolved prior to further clinical studies.

The main factors that are considered important when attempting to make the selection of a treatment dosage for therapeutic ultrasound are frequency (ter Haar 1987), output intensity (Young and Dyson 1990), duration of exposure (Oakley 1978), and the depth of target tissue (Wells 1977, Robertson and Ward 1997). When considering all these factors as a whole, the therapist attempts to choose the most appropriate machine parameters (frequency, intensity and duration), which have the most likely chance of enabling the ultrasonic energy to reach the target tissues. The nature and depth of the target tissues are estimated from the therapist's knowledge of anatomy, pathology, and physiology. Therefore, in order for therapists to select an appropriate treatment dosage based on this rationale, two main questions need to be addressed:

- How to deliver the required amount of ultrasonic energy to the target tissues consistently?
- How much ultrasonic energy is needed to effect the various biophysical changes in the target tissues?

The first question precedes the second question, and is the main focus of this thesis. The second question can only be answered by conducting various clinical trials, as well as cellular and animal studies, based on the answers derived from the first

question. The answers to both questions, however, are required for a complete dosimetry model.

There are several parts to the first question and for clarity, it will be broken down as follows:

- What is the relationship between ultrasonic frequency and amount of heating at the various target depths?
- What is the relationship between ultrasonic intensity and amount of heating at the various target depths?
- What is the relationship between duration of exposure to ultrasound and amount of heating at the various target depths?

In order for any useful model to be derived from these studies, the data must be complete. Hence for the frequency factor, data must be available for at least 1 MHz and 3 MHz as they are the most commonly used frequencies in therapeutic ultrasound. For the intensity factor, the full range of intensities ranging from the minimum to the maximum machine allowable, or up to the maximum intensity before tissue damage begins to occur, should be investigated. For the duration factor, data for at least 10 minutes exposure to ultrasound should be available as this represents the maximum standard treatment time in most clinical applications (McDiarmid and Burns 1987). Finally, the maximum depth of the target tissue to be investigated should be sufficient to account for all the possible target tissues encountered in most clinical applications. From past investigations, the depth of target tissue investigated ranged from 3 cm (Levine et al 2001) to 5 cm (Draper et al 1995a) to 15 cm (Ward and Robertson 1996), depending on the experimental animal chosen for the study, as well as other factors. Based on anatomical considerations, an appropriate depth for a human model or an equivalent animal model (such as pigs) would be about 5 cm (Draper et al 1995a).

Unfortunately, studies investigating the relationships between frequency, intensity and duration of exposure on the amount of heating at the various target depths are incomplete for at least one or more factors. For example, Lehmann et al (1967a, 1968) investigated only one intensity (1.5 Watts/cm^2) for five minutes exposure to ultrasound, whereas Levine et al (2001) and Draper et al (1995a) investigated two intensities (1.0 and 1.5 Watts/cm^2) and four intensities (0.5, 1.0, 1.5 and 2.0 Watts/cm^2) respectively, for ten minutes exposure.

In addition to these sub-questions, and implicit in the main question is the issue of consistency in delivery of the energy to the target tissues. That is, what are the factors that affect reproducibility and reliability of the ultrasonic treatment procedure? Some of these factors have been identified as follows:

- Direct (in-contact) versus indirect technique (e.g. underwater): Which is the more reproducible and reliable method? The direct (in-contact) technique has been demonstrated to be more consistent and effective by several authors (Balmaseda et al 1986, Forrest and Rosen 1992, Draper et al 1993, Robertson and Ward 1996).
- Angle of application of the transducer: What is the optimum angle between the transducer and the skin? Kimura et al (1998) highlighted the importance of maintaining a perpendicular incident angle between the flat surface of the transducer and the target tissue in order to achieve consistency in treatment applications to body contours that are not naturally flat.
- Temperature of the coupling gel: What is the optimum temperature for the coupling gel? Previous studies have demonstrated that coupling gel at room temperature was preferable to both cooled and heated gel (Lehmann et al 1966a, Oshikoya et al 2000).
- Pressure of the transducer on the skin: What is the optimum pressure to apply to

maintain contact between the transducer and the skin? Klucinec et al (1997) demonstrated that increased pressure of the transducer on the skin had an effect on acoustic transmissivity. To maintain consistency in treatment applications, a standard pressure (such as the weight of the transducer) should be applied.

- Size of the treatment area: What is the ideal size for the treatment area? Chan et al (1998) recommended that a small treatment size was more effective and provided longer heating than a large treatment size. However, the study by Chan et al (1998) only investigated one frequency (3 MHz). It is unclear if the same relationship exists for 1 MHz.
- Speed of movement of the transducer: What is the most appropriate speed for moving the transducer? It is unclear as to what is the most appropriate speed for moving the transducer as no studies addressing this issue were found.

Each of these questions will be further elaborated upon in Chapter 2, the Literature Review. Without the issue of reproducibility and reliability of the treatment procedure being ascertained, the main question ("How to deliver the required amount of ultrasonic energy to the target tissues?") cannot be answered with certainty.

Finally, in order to fully clarify the issue of "required amount of ultrasonic energy", it is necessary to ascertain if there are any limits that may exceed this "required amount". Specifically, there is a need to identify the upper safety limits when attempting to deliver the "required amount of ultrasonic energy". Again for clarity, this can be broken down as follows:

- What is the maximum machine allowable intensity of ultrasonic energy that can be applied without causing irreversible thermal and mechanical cell injury?

The experimental models that have been adopted in the past to answer some of these questions include an *in-vitro* pig model (Gammell et al 1979, Goss et al 1979, Forrest and Rosen 1992, Robertson and Ward 1996, Ward and Robertson 1996,

Klucinec et al 1997), an *in-vivo* pig model (Lehmann et al 1967b, Lehmann et al 1968), an *in-vivo* dog model (Levine et al 2001), a phantom-tissue model (Kimura et al 1998), and an *in-vivo* human model (Draper et al 1995a, Oshikoya et al 2000). The choice of *in-vivo* versus *in-vitro* depends largely on the type of instrumentation used to measure the increase in temperature at the various depths. For *in-vivo* studies, temperature sensors such as probes are required. However, the exact location of the probe cannot be ascertained without some form of diagnostic imaging instruments (such as x-rays or diagnostic ultrasound). Another disadvantage is that the probes themselves are subjected to heating by the ultrasonic energy and this may affect the accuracy of the data obtained. For *in-vitro* studies, however, these disadvantages can be easily overcome by using non-contact temperature sensors such as infra-red thermography which provides full access to the cross-section of the tissues as they are exposed to the ultrasonic energy. The main shortcoming with this technique, however, would be the inability to account for the influence of superficial and deep circulation on the increased temperatures at various depths. Ideally, the experimental animal chosen for these types of studies should be human. However, because of major ethical considerations, and the fact that the safety of the subjects cannot be assured, an alternative animal model that closely resembles the human model in terms of fat, muscle and bone composition can be considered. The pig model meets all these requirements (Lavker et al 1991) and has been used successfully in the past for similar investigations (Forrest and Rosen 1992, Robertson and Ward 1996, Ward and Robertson 1996, Klucinec et al 1997). Hence, an *in-vitro* pig model using infrared thermography would be a suitable experimental model for the proposed investigations.

1.2 SUMMARY

The purposes of this study are:

a. To clarify the effects of the various dosage parameters (frequency, intensity and duration of exposure) on heating at various depths up to 5 cm,

b. and to propose an evidence-based model for determining treatment dosages based on delivering the required amount of ultrasonic energy to the target tissues.

A series of studies were set up to examine the following (in sequence):

a. What is the effect of varying the movement speed (slow, moderate and fast) of the transducer on depth of heating?

b. What is the effect of varying the size of the treatment area (small, medium and large) on the depth of heating?

c. What is the maximum output intensity that can cause irreversible thermal and mechanical cell damage?

d. What is the effect of varying the frequency of the ultrasonic generator, output intensity and duration of exposure on the depth of heating?

All laboratory work was carried out in the Department of Physical Therapy at Shinshu University in Matsumoto, Japan. Funding was made available partly from a three-year research grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan (Research Grant No. 12832020-00), and partly from an International Postgraduate Research Scholarship from Curtin University of Technology, Perth, Australia. The Animal Research Ethics Committees of Curtin University (E29/2000) and Shinshu University approved the study prior to its commencement.

CHAPTER TWO

LITERATURE REVIEW

2.1 WHAT IS THERAPEUTIC ULTRASOUND?

When an alternating current is applied to piezoelectric transducers such as quartz or synthetic ceramics e.g. lead zirconate titanate (PZT), mechanical wave motions corresponding to a certain frequency are generated (ter Haar 1987). In therapeutic ultrasound, the frequencies usually employed are from 0.5 MHz to 5 MHz (ter Haar 1987). When the transducer is in contact with a liquid or solid, it transmits energy away from the source, causing molecules in its path to be set into oscillation, thereby imparting its energy from molecule to molecule (Williams 1987). In this manner, energy from the source is gradually transmitted to and absorbed by the medium it travels through, assuming that the medium is homogeneous. The mechanical energy being absorbed is thereby converted into thermal energy, causing an increase in temperature in the medium. The increase in temperature due to absorption of the ultrasonic energy is related to the ultrasound frequency, intensity and duration of the ultrasound exposure (Williams 1987). In human tissues, Williams (1987) estimated the rate of increase in temperature for most soft tissues produced by a 1 MHz ultrasound frequency at an intensity of 1 W/cm^2 is about $0.86^{o}C$ per minute.

In non-homogeneous media, the energy can be reflected or refracted at the boundary between different media (Williams 1987). The extent of reflection or refraction depends on the difference in acoustic impedance at the boundary. The

acoustic impedance of a material is defined as "a characteristic number obtained by multiplying the density of the material by the speed at which sound travels inside it" (Williams 1987, p. 114). The greater the difference in the acoustic impedance at the boundary the greater will be the reflection away from the boundary, and correspondingly the smaller the refraction across the boundary (Williams 1987). Conversely, the smaller the difference in the acoustic impedance at the boundary, the smaller the reflection away from the boundary and the greater the refraction across the boundary. The difference in acoustic impedance between most soft tissues such as skin, fat and muscle is small, for example, the reflection at skin / fat or fat / muscle interface is only about 1%. In contrast, the difference in acoustic impedance between soft tissue and hard tissue (such as bone) is greater, and hence, at the soft tissue / bone interface, about 15% to 40% of energy can be reflected away from the interface (Williams 1987).

In order to minimize loss of energy from the transducer to the tissue, a suitable coupling medium with acoustic impedance similar to that of soft tissue is used (Williams 1987). Degassed water is an excellent coupling medium, but because of its low viscosity, it is not suitable for all types of clinical applications. Commercially available gels are ideal for most applications (Williams 1987).

Most ultrasonic generators are capable of providing either a continuous output from the transducer or an interrupted output (commonly known as pulsed mode) (Low and Reed 2000). The interrupted output is described by using a mark:space ratio, or a duty cycle (Low and Reed 2000). The mark:space ratio indicates the duration that the pulse wave is on, followed by the duration when it is off. Hence, a mark:space ratio of 1:4 would mean that the pulse wave is on for 1 ms and off for 4 ms. Duty cycle is the ratio of the pulse on duration to the duration of the entire pulse (on + off), and is expressed as a percentage. In the example

above, a mark:space ratio of 1:4 would have a duty cycle of 20%.

The energy from the transducer is usually expressed in terms of intensity or power (Low and Reed 2000). Intensity is the amount of energy per unit area, and is usually expressed in Watts/cm^2 (Low and Reed 2000). For simplicity, the surface area of the transducer head is used and this typically ranges from 1 cm^2 to 5 cm^2. For more accurate measurements, the effective radiating area (ERA) of the transducer is used, and this is measured using a hydrophone (Low and Reed 2000). Average intensity is taken over space (spatial average) or over time (temporal average), or both. Space averaged intensity is the intensity of ultrasound that has been averaged over a specified area such as the surface area or the ERA of the transducer (Low and Reed 2000). This space-averaged intensity is also commonly referred to as space averaged, temporal peak (SATP). Time averaged intensity is only applicable in the pulsed mode (Low and Reed 2000). The most common time averaged intensity is the spaced averaged, time averaged (SATA). As the name suggests, this is the average intensity taken over both a specified surface area as well as a specified time. Power, on the other hand, is the total energy in the ultrasonic field across the entire surface area of the transducer (i.e. intensity multiplied by surface area or ERA), and is usually expressed in watts (Low and Reed 2000). Total power is the power multiplied by the duration of exposure (Low and Reed 2000) and is usually expressed in joules.

Finally, the duration of exposure or time of irradiation is the total time that the tissues are exposed to the ultrasonic energy and is usually provided by an internal clock display on the machine (Low and Reed 2000). For pulsed mode applications, the actual time of irradiation is the time multiplied by the duty cycle (Low and Reed 2000).

2.2 USAGE OF THERAPEUTIC ULTRASOUND

Therapeutic ultrasound is a commonly used treatment modality or electrophysical agent employed by physiotherapists in the treatment of acute and chronic pain conditions, for the control of inflammation and swelling, for increasing tissue extensibility, and for improving tissue healing (McDiarmid and Burns 1987, Low and Reed 2000). However, there is no consensus among physiotherapists about the optimum treatment dosages for any particular condition (Partridge 1990).

Several studies of usage of ultrasound among physiotherapists have been reported (Robinson and Snyder-Mackler 1988; Lindsay et al, 1990, 1995; Pope et al 1995). Most of these studies, however, reported the frequency of usage of therapeutic ultrasound in various clinical settings in different countries. To date, there is an absence of information about the users of ultrasound regarding their current level of knowledge as well as their opinions on treatment dosages for various conditions commonly treated with ultrasound.

To examine common usage among physiotherapists, a questionnaire survey was conducted which included 147 physiotherapists in Singapore and this elicited a response rate of 66.7% (98 respondents) (Goh et al 1999). The survey had three aims: (a) to document the current status of usage of therapeutic ultrasound in Singapore, (b) to establish the current level of knowledge among users of therapeutic ultrasound, and (c) to sample opinions on what constitutes an appropriate treatment dosage for various specified conditions.

Only 35% of these respondents reported that they used ultrasound at least once per day, and this compares less favorably with that reported in other countries. For example, figures available for Australia (93%) (Lindsay et al 1990), Canada (93%) (Lindsay et al 1995), United States (79%) (Robinson and Snyder-Mackler

1988), and United Kingdom (88%) (Pope et al 1995) indicate much higher usage in all these countries. However, this difference could be partially accounted for by the different target populations in each study and the preferential usage of electrophysical agents within these groups. For example, Lindsay et al (1990, 1995) and Robinson and Snyder-Mackler (1988) surveyed private practitioners working in an outpatient setting, whereas Pope et al (1995) surveyed physiotherapists working in outpatient departments of public hospitals. These groups of physiotherapists are generally perceived to be the main users of electrophysical agents within the physiotherapy profession. In contrast, Goh et al's survey (1999) targeted everyone in the profession, regardless of their specialty. When the usage rate of the outpatient physiotherapists in Singapore were analyzed separately, the once a day usage rate for therapeutic ultrasound increased to 59.5%. Despite the target population now being similar, this figure still compares less favorably than that reported in other countries. However, the most recent survey on ultrasound usage reported in the literature was conducted in 1995 (Lindsay et al 1995, Pope et al 1995). It is possible that Goh et al's survey (1999) conducted 4 years later, could be an indication that there has been some decline in the use of ultrasound in the intervening period perhaps as a consequence of the various unfavourable reviews on therapeutic ultrasound published since 1995 (Gam & Johannsen 1995, Feine & Lund 1997, Hay-Smith & Reed 1997, van der Heijden et al 1997, Johannsen et al 1998, van der Windt et al 1999). Despite this, 78% of respondents reported that ultrasound was effective based on their own experience, but only 42% indicated that they possessed adequate knowledge for determining treatment dosage for the various conditions (Goh et al 1999).

An overwhelming 92% of respondents suggested that there was not enough evidence to guide them in determining treatment dosages. This concern was

supported by poor consensus among respondents on treatment dosages for the specified medical conditions. When respondents were asked their opinions about appropriate treatment dosages for seven specific medical conditions, the majority stated that they considered the pulsed mode to be appropriate for the acute phase and the continuous mode for the chronic phase. The nature of the problem (acute or chronic), the depth of the target tissue (superficial or deep) and the size of the treatment head (small or large) were considered to be the three most important factors when determining the treatment dosage. The respondents' choice of treatment intensity varied from 0.66 Watts/cm^2 (for acute lateral epicondylitis) to 1.03 Watts/cm^2 (for chronic torn quadriceps). In the determination of the treatment intensity, males consistently chose higher treatment intensities than females ($p<0.001$); the geriatric and pediatric practitioners favored higher treatment intensities compared with the other specialties ($p=0.026$); and graduates from the United Kingdom were more conservative, choosing lower treatment intensities than graduates from other countries such as Australia and Singapore ($p<0.001$).

It appears from the results of this survey (Goh et al 1999) that the determination of treatment dosages for therapeutic ultrasound requires further clarification. In the absence of concrete guidelines based on experimental data, clinicians' selection of treatment dosage appears arbitrary (Reid and Cummings 1973, Goh et al 1999) and may in fact, differ according to factors such as gender, clinical specialty, country of training and educational background (Goh et al 1999).

Gam and Johannsen (1995), in their review of 22 randomized controlled trials, were unable to demonstrate a dose-response relationship due to a lack of adequate treatment details provided in the studies. A more recent review of 24 randomised controlled trials in therapeutic ultrasound (Robertson 2002) was also unable to identify a relationship between the dosage and outcomes. Robertson

(2002) cautioned that there appears to be no justification for the currently favoured dosage parameters used in therapeutic ultrasound treatment. The lack of a common approach to the determination of treatment dosages could be a significant factor affecting the outcome of most clinical trials. To ensure the value of future clinical trials, it would appear that an internationally accepted approach for the determination of treatment dosages needs to be developed.

In any treatment using therapeutic ultrasound, the first consideration is to provide sufficient energy to the target tissue to be effective, without exceeding the amount where damage to tissues occurs or where the condition itself becomes exacerbated. The second, equally important consideration is the ability to deliver the same dose of ultrasonic energy to the target tissues consistently during each treatment session both within and between patients. Both these considerations require measurement of the amount of ultrasonic energy being emitted at any given instrument setting. Williams (1987) suggested that the measured parameter should be the same parameter responsible for producing the biological effects within the patient's tissues. The physical mechanisms responsible for producing the biological effects of ultrasound can be classified as thermal and non-thermal mechanisms (Dyson 1987). While temperature is a useful measure of the thermal effects non-thermal effects are not sufficiently understood and their assessment is not currently possible.

Non-thermal mechanisms are usually enhanced by delivering the ultrasound energy in a pulsed mode. Pulse mode, however, does not imply that there is an absence of heating in the tissues. On the contrary, depending on other factors such as intensity and duration of exposure, pulsed ultrasound, in some instances, can produce more heating than continuous ultrasound (Williams 1987). Both the thermal and non-thermal mechanisms can be measured by the amount of heating

produced within the tissues (Williams 1987) as ultrasound is transmitted through, and subsequently absorbed by the tissues. Therefore, in order to provide a common model to assist clinicians and researchers to determine treatment dosages in therapeutic ultrasound, a thorough understanding of the relationship between the ultrasound parameters such as frequency, intensity, and duration of exposure, and the distribution of heat within the tissues is necessary. Therefore, the following sections will elaborate on three main points:

a. The factors to be considered in determining treatment dosages in therapeutic ultrasound (Section 2.3, page 17);

b. Those factors which affect the consistency of delivery of ultrasonic energy to the target tissues during each treatment session (Section 2.4, page 26);

c. The relationship between the frequency, intensity and duration of ultrasound energy delivered and the pattern of heat distribution within the tissues (Section 2.5, page 36).

2.3 FACTORS TO CONSIDER IN DETERMINING TREATMENT DOSAGE FOR THERAPEUTIC ULTRASOUND

The relevant parameters that require careful selection and consideration when attempting to decide on a treatment dose for therapeutic ultrasound can be divided into 3 main categories: machine parameters; target tissue characteristics, and machine-target tissue interface.

2.3.1 Machine Parameters:

a. Frequency:

Although the common frequencies in therapeutic ultrasound range from 0.5

MHz to 5.0 MHz (ter Haar 1987), manufacturers of contemporary ultrasound machines generally provide a choice of either 1 MHz or 3 MHz. The choice of frequency is selected according to the depth of tissue to be treated. Since high frequencies attenuate more rapidly than lower frequencies (ter Haar 1987), the 1 MHz ultrasound frequency is considered to provide heating for deep tissues, whereas the 3 MHz is considered to provide heating for superficial tissues (Low and Reed 2000). However, the exact nature of this purported difference needs to be clarified by investigating the relationship between frequency and increase in temperature at various distances from the skin surface through subcutaneous fat to deep muscle. Some of this information is already available from current literature, and will be discussed in detailed in section 2.5 (page 36).

b. Intensity:

The biophysical effects of ultrasound are also intensity dependent; too low a dose will have no effect and too high will be damaging (Young and Dyson 1990). The magnitude of intensity that is too low or too high is not clear. MacDonald and Shipster (1981) claim that there is insufficient information concerning the temperatures reached in different tissues, and the extent to which these may be at levels capable of damaging the tissues.

It has been estimated that at 1 MHz ultrasound frequency, 1 Watt/cm^2 would be required to raise the temperature of most soft tissues by 0.86°C per minute, in the absence of heat removal by local circulation (Williams (1987). Very little has been written on the effect of local circulation on heat removal and the overall cooling effect likely to occur in the presence of normal circulation. In fact, Lehmann et al, (1966b) appears to be the only report available. Lehmann et al (1966b) investigated the effect of a tourniquet applied proximal to the treatment area. Temperature changes were recorded during treatment prior to application of a tourniquet, followed

by a brief period when circulation was interrupted with the tourniquet, and again following removal of the tourniquet. In the presence of local circulation, the overall cooling effect of circulation on the heating pattern is estimated to be about $-1^{\circ}C$ to $-3^{\circ}C$ (Lehmann et al 1966b). While there have been several more recent in-vivo studies (Draper and Sunderland 1993, Draper et al 1993, 1995b, 1998a, Rimington 1994, Myrer et al 2001) where circulation was present in the subjects, none of these studies examined the effect of circulation on the temperature increase.

Therapeutic effects are said to occur at temperatures between $40^{\circ}C$ and $45^{\circ}C$, maintained for at least five minutes (Lehmann and deLateur 1982). Dyson (1987) suggests that therapeutic thermal effects can be produced using doses of 0.5 to 3.0 Watts/cm^2 and non-thermal effects have been reported at 0.1 and 0.2 Watts/cm^2. The higher doses required for effective heating have been identified as possibly damaging (Dyson 1987). However, supporting data for these recommendations were not provided by the author (Dyson 1987).

Unfortunately, the intensity output of many ultrasound machines used in the clinical setting and experimental trials has been shown to be unreliable (Allen and Battye 1978, Chapman 1986, Docker 1987, Lloyd and Evans 1988). In each of these surveys, it was found that between 48% and 71% of machines examined were beyond the acceptable range of error, with lower intensities being most susceptible to greatest inaccuracies (Lloyd and Evans 1988). The issue of machine reliability will be discussed in further detail in section 2.4 (page 26).

Verification of the relationship between intensity and increase in temperature at the target tissue is required to provide support for the guidelines proposed by Williams (1987) and Dyson (1987). Furthermore, the minimum intensity at which damage to the target (and intervening) tissues may occur must also be determined. This could be achieved by exposing tissues to a range of intensities

and examining them microscopically for cellular damage. No such studies have been reported to date.

c. Duration of exposure:

The duration of exposure to ultrasound for any treatment episode is generally determined by setting the timer built into the machine. In the absence of concrete guidelines, most clinical studies employ durations of between three and 10 minutes (McDiarmid and Burns 1987).

Oakley (1987) recommended that the duration of exposure should be related to the size of the treatment area; that is, one to two minutes for each area corresponding to 1.5 times the size of the transducer. Oakley (1987) also recommended that the duration of exposure could be increased by half-minute intervals up to a maximum of three minutes, if the patient is improving. Similarly, Reid and Cummings (1973) suggested that the duration prescribed should be based on an area twice the size of the treatment head. None of these recommendations, however, were supported by any experimental evidence.

Based on Lehmann and deLateur's (1982) claim that therapeutic effects occur at temperatures between 40°C and 45°C maintained for at least five minutes, and allowing for at least two or three minutes for the baseline pre-treatment temperature to reach the target temperatures, it would appear that a treatment duration of at least seven or eight minutes would be appropriate. However, unless the relationship between duration of exposure and increase in temperature for the entire range of intensities and frequencies is clarified, none of these recommendations can be verified. Some of this information is already available from current literature, and will be discussed in more detail in section 2.5 (page 36).

d. Duty cycle

First generation therapeutic ultrasound machines available in the early 1950s

and 1960s did not provide for anything other than a continuous mode of delivery. Pulsed mode ultrasound was introduced much later (Oakley 1978) and along with this feature, the mark:space ratio or duty cycle was introduced to define the exact nature of the pulsed mode. In thermal applications where heating is the primary objective, the amount of energy to be delivered to the target tissue can be adjusted by varying the intensity (for continuous and pulsed mode) or by varying the duty cycle (for pulsed mode). However, having two variables to control the desired amount of energy adds unnecessary complexity. The simplest option is to maintain the duty cycle at 100% (i.e. continuous mode) and to adjust the intensity in order to deliver the desired amount of energy to the target tissue. Even in cases when excessive heating is not required, this method of adjusting the intensity may still be appropriate.

The duty cycle parameter, therefore, may not be a necessary factor for determining treatment dosage as it can be kept at 100% and the desired amount of energy to be delivered could be controlled by the intensity factor alone. It is interesting that the pulsed mode was offered as an afterthought and was not included in the original design of the machine. The rationale for including the pulsed mode has never been adequately explained or justified, and may have been responsible for complicating the issue of dosimetry in therapeutic ultrasound. If the objective in selecting a dosage is to deliver the appropriate ultrasonic energy to the target tissues, the duty cycle parameter may prove to be irrelevant as this can be achieved adequately by varying the intensity.

e. Summary

The machine parameters that can be considered important factors for the determination of treatment dosage for therapeutic ultrasound are frequency of the transducer, output intensity, and duration of exposure. For thermal effects, the

continuous mode provides maximal heating and hence the pulsed mode may be considered as irrelevant in thermal studies. The relationship between temperature increase at various target distances from the skin surface and frequency, output intensity and duration of exposure requires thorough clarification in order to formulate clear treatment guidelines for dosimetry in therapeutic ultrasound. Furthermore, it is also necessary to determine the minimum intensity at which irreversible thermal injury to cells occur.

2.3.2 Target Tissue Characteristics

a. Depth of target tissue

As the ultrasound energy passes through the body's tissues, attenuation of the energy occurs with loss of energy as the tissue depth increases due to absorption and scattering (ter Haar 1987). Wells (1977) defined attenuation as the propagation loss of ultrasonic energy traveling through a medium so that its intensity is reduced as a function of distance. The frequencies between 0.8 and 3.0 MHz have been found to be most therapeutically useful due to their attenuation properties (Fyfe and Bullock 1985). There are two methods for describing this loss of energy from attenuation: by penetration depth, or by half value distance. According to Ward (1986) ultrasonic energy attenuates exponentially with depth, and is reduced by 63% each time the wave passes through a distance of one penetration depth. Wells (1977) has used a half-value distance to describe the same phenomenon, that is the tissue depth at which ultrasound is reduced by 50%. At 3 MHz, the half value distance is 1.5 cm, and at 1 MHz the half value distance is about 5 cm (Wells 1977). Therefore, in order to fully appreciate the relationship between frequency and intensity on the heating pattern of the tissues, measurements of temperature increases for 3 MHz and 1 MHz should cover minimum depths of 1.5 cm and 5 cm

respectively (Draper et al 1995a, Wells 1977). Most clinical applications, however, can be adequately covered up to a maximum depth of 5 cm, and this may be considered as an appropriate depth of target tissue for subsequent investigations.

b. Composition of target tissues

Clinical applications of therapeutic ultrasound involve several different types of target tissues including muscle, ligaments, tendons, joint capsule and even bone (McDiarmid and Burns 1987). With most ultrasound applications, the ultrasonic energy is transmitted across a non-homogenous medium consisting of the skin, subcutaneous fat, muscle and bone.

When an ultrasonic wave encounters a boundary between two different tissue types, some of the wave is reflected at this boundary, while the rest is refracted across the boundary according to the extent of mismatch in acoustic impedance of the two types of tissues (Williams 1987).

In order to investigate the heating pattern of ultrasound at various intensities and frequencies, it is desirable to avoid instances where sudden mismatch in acoustic impedance can confound the data. Therefore, it might be necessary for initial investigations of the heating pattern to be carried out for soft tissues only, without the presence of any bone. Subsequent investigations could then examine the influence of the presence of bony tissue, and how this might affect the basic heating pattern found in soft tissues. Again, there are some available data on the influence of bone on the heating pattern (Lehmann et al 1967a, 1967b, 1968) but it is difficult to make sense of the data without the pre-requisite information on the basic heating pattern in soft-tissues.

It has been suggested that the different tissue types attenuate ultrasound to varying degrees, depending on their acoustic impedance and the frequency of the ultrasound (Allen and Battye 1978, Williams 1987). Tissues with high protein

content, such as muscle and tendon, are said to absorb more ultrasonic energy than those with high fat content (Dyson 1987). While the skin barrier is quite consistent, the amount of subcutaneous fat varies from person to person. Since the ultrasonic energy inevitably has to travel through the skin and subcutaneous fat before reaching its target tissue, the variability of fat thickness could influence the level of heating for more deeply sited tissues. Draper and Sunderland (1993) investigated the effect of varying fat thickness on the level of heating in the gastrocnemius muscle at a constant depth of three centimeters subcutaneously. Their subjects were 20 healthy males with an average of 12.6 ± 6.1 mm of fat (range of 4 to 30 mm) as measured by skinfold calipers. Increase in temperature was recorded from a thermistor probe inserted into the belly of the gastrocnemius muscle at a depth of 3 cm from the surface. A 1 MHz therapeutic ultrasound with an intensity of 1.5 Watts/cm^2 (continuous mode) was applied for 10 minutes on the skin overlying the target muscle tissue in a regular stroking manner. The mean temperature increase recorded at the 3 cm deep site was 4.9°C. Their results demonstrated that there was no significant correlation between fat thickness and tissue temperature rise at 3 cm depth. The amount of fat (within the range measured in their subjects) did not affect the amount of heating observed in the deeper tissues. Hence, a variation of fat thickness up to 3 cm would not be a confounding variable in any investigations into the relationship between ultrasound dosage parameters such as frequency, intensity and duration on the heating pattern distribution.

c. Summary

The target-tissue characteristics that can be considered important factors for the determination of treatment dosage for therapeutic ultrasound are depth (distance from skin surface) and composition of target tissue. In order to fully appreciate the relationship between frequency, output intensity and duration of exposure on the

heating pattern of the target tissues, measurements of temperature increase for 3 MHz and 1 MHz should cover a maximum depth of 5 cm in order to account for all the possible target tissues in most clinical applications. In addition, a variation of fat thickness up to 3 cm would not be a confounding variable in the heating pattern of the specimens exposed to therapeutic ultrasound.

2.3.3 Machine – Target Tissue Interface

a. Transmissivity of coupling agent

The transducer-skin interface is usually air-filled (Williams 1987). Since the passage of ultrasound waves through a medium depends on the acoustic impedance of the medium, and reflection occurs at the boundaries between different media, it has been reported that 99.9% of ultrasound energy will be reflected away from the dry skin (Williams 1987). In order to overcome such a high percentage of reflection of the ultrasonic energy at the transducer-skin interface, a coupling agent is usually used. Reid and Cummings (1973) investigated the transmissivity of different types of coupling agents (air, mineral oil, cardio-cream, distilled water, glycerol, and aquasonic gel). They reported that aquasonic gel had the highest percentage of transmissivity (72.6%). Since then, other investigators have reported similar or higher transmissivity for aquasonic gel of between 89% to 98% (Benson and McElnay 1988, Klucinec et al 2000). Aquasonic gel appears to be the preferred coupling agent for most therapeutic ultrasound applications today and should therefore be the coupling agent of choice for any investigations employing a direct contact technique (see Section 2.4.2, page 29).

b. Summary

The machine-target tissue interface parameter that can be considered an important factor for the determination of treatment dosage for therapeutic ultrasound

is the transmissivity of the coupling agent. The transmissivity of aquasonic gel has been demonstrated to be consistently high and is the preferred coupling agent for most studies.

2.4 FACTORS AFFECTING THE CONSISTENCY (REPRODUCIBILITY AND RELIABILITY) OF DELIVERING THE SAME DOSE OF ULTRASONIC ENERGY TO THE TARGET TISSUES DURING A THERAPEUTIC ULTRASOUND TREATMENT SESSION

Implicit in any proposed model for determining treatment dosages is the ability to consistently deliver the same amount of ultrasonic energy to the target tissues for every treatment episode for the same patient, and even for different patients.

The factors that can affect the consistency of a therapeutic ultrasound treatment episode may be categorized into two groups: machine-related factors, and protocol-related factors.

2.4.1 Machine-related factors

a. Reliability of output intensity

Widespread calibration inaccuracies found in the output intensity of therapeutic ultrasound machines may have affected the progress and validity of clinical trials in the field (Fyfe and Bullock 1985). Perhaps this factor alone represents the greatest threat to the consistency of a therapeutic ultrasound application due to its high prevalence and ability to remain undetected, despite increasing awareness of the problem.

The International Electrotechnical Commission (IEC) has as its mission the promotion of international cooperation on all questions of electrotechnical standardization and related matters, such as the assessment of conformity to standards in the fields of electricity, electronics and related technologies. The power output performance standard, specified for therapeutic ultrasound by the IEC is described in IEC 61689 (1996). It states that the ultrasound power output displayed on the machine must be accurate within ±10% of the real output of the device. This replaces the older recommendation (IEC 60150, 1963) of ± 20% error between the display on the machine and real output of the device. Similarly, the Food and Drug administration in the United States of America (FDA) currently recommends that the error in power output for therapeutic ultrasound shall not exceed ± 20% (FDA 1997, 1050.10 21CFR , Ch 1, 4-1-97 edition, pp 580-583).

Unfortunately, most therapeutic ultrasound devices do not comply with the recommended standard. However, the fault is not necessarily with the manufacturers, but may be with users who demonstrate inadequate understanding of the importance of proper calibration procedures (Stewart et al 1974, Guirro et al 1997).

Stewart et al (1974), surveyed 58 therapeutic ultrasound units used by physiotherapists in Florida, USA and found that almost all 58 units exceeded ± 20% error in their power output. The error on one particular unit was as high as + 900%. More recently, Guirro et al (1997) surveyed 31 therapeutic ultrasound units that were being used on a daily basis for treatment in Sao Paulo, Brazil. They concluded that almost all the equipment tested was found to emit energy greater than ±30% of the machine intensity display for both pulsed and continuous modes. Three of the 31 machines surveyed, did not emit any radiation, regardless of the intensity indicated on the panel (Guirro et al 1997). Similar results were found from surveys in

Australia (Thomson and Fyfe 1983), Canada (Snow 1982) and the United Kingdom (Pye and Milford 1994).

Therefore, the results of any clinical trials that fail to report calibration of the ultrasound machine prior to, and during the course of the study, must be considered suspect in view of the reported errors in uncalibrated ultrasonic devices.

b. Performance stability

The recommended guideline from the Australian and Canadian governments regarding frequency of calibration of ultrasound units is at least once per year (Rivest et al 1987). This guideline assumes that the performance of an ultrasound machine is sufficiently stable not to warrant frequent calibration. Rivest et al (1987) however, challenged this assumption and examined the performance stability of 29 ultrasound units from 11 physiotherapy departments in Montreal, Canada. The power output, timer accuracy, and ultrasonic frequency of all ultrasonic units were verified every 4 weeks for 20 weeks, followed by two more verification visits on the 32^{nd} and 44^{th} week (12 week intervals). During the initial evaluation, 66% of the ultrasound machines were found to be outside the $\pm$20% error margin for power output and, therefore, required calibration. For the duration of the study (44 weeks), 62% of the ultrasound machines required calibration at least twice, 38% at least three times, and 31% at least 4 times. Based on the results of their study, Rivest et al (1987) recommended that ultrasound machines should be calibrated at least once every four weeks, and at even shorter intervals for some units that were heavily used.

Rivest et al (1987) demonstrated that the performance stability of the ultrasound unit could not be assumed. Moreover, for clinical trials, the usual practice of calibrating the ultrasound unit just prior to the study may not be sufficient, if the performance stability of the unit is unknown and the machine will be in use for more than 4 weeks.

Summary

The machine-related factors that can be considered important factors for the consistency of delivering the same dose of ultrasonic energy to the target tissues during a therapeutic ultrasound treatment session are reliability of the output intensity and the performance stability of the ultrasonic device. The output intensity of most therapeutic ultrasound devices had been shown to be unreliable. Therefore, it is necessary to perform calibration of therapeutic ultrasound devices prior to any study. Furthermore, if the period of study extends for more than four weeks, it is necessary to perform calibration of the therapeutic ultrasound devices at a minimum of 4-weekly intervals.

2.4.2 Protocol-related factors

a. Technique of application:

Two common techniques of application have been advocated: direct and indirect technique (Low and Reed 2000). The direct technique involves actual contact between the applicator and the surface of the skin, with a thin layer of suitable coupling medium in-between. The indirect technique involves no contact between the applicator and the surface of the skin, with a varying thickness of coupling medium in-between. The coupling media used for indirect application include water (subaqueous), oil, silicon, gel pads, and gel or water filled bladders.

Differences in deep tissue heating from the direct and indirect (subaqueous) contact methods had been investigated in a post-mortem pig model (Robertson and Ward 1996). The pig specimens were exposed to ultrasound (1 MHz, 1 Watt/cm^2, 20 minutes, stationary head) under five conditions: direct (gel) and indirect (subaqueous) contact at distances of 0, 1, 2, and 4 cm between applicator and skin. Increases in temperatures were recorded at six tissue depths for each of the five

conditions during exposure to ultrasound. The direct contact (gel) method provided the greatest amount of heating at all six tissue depths compared with the indirect (subaqueous) contact method. Furthermore, increasing the distance from the transducer to the skin in the subaqueous method resulted in significantly less heating in the tissues. When compared with the 0 cm distance (100% heating), the amount of heating was reduced to 69% at 1 cm, 56% at 2 cm, and 44% at 4 cm distance. The authors concluded that the direct (gel) contact technique was not interchangeable with the indirect (subaqueous) contact technique, and that the former was better at producing deep tissue heating.

However, it can be argued that using a post-mortem pig experimental model does not permit evaluation of the effects of local circulation on the tissue heating caused by exposure to ultrasound. This limitation can be overcome by using an *in vivo* animal model. In a similar experiment, Forrest and Rosen (1992) compared the deep tissue heating effects of ultrasound treatment using direct (gel) with an indirect (subaqueous) contact technique in live pigs. Tissues were exposed to ultrasound at 1 MHz, 2.5 Watts/cm^2, for 10 minutes using a moving transducer technique. The indirect (subaqueous) contact technique was carried out using degassed distilled water, with a distance of 1.5 cm from the skin to the transducer. Forrest and Rosen (1992) also concluded that the direct (gel) contact technique provided more effective heating than the indirect (subaqueous) contact application.

Similar conclusions were drawn from a comparable study, using an *in vivo* human model (Draper et al 1993). The gastrocnemius muscles of 20 healthy subjects were exposed to ultrasound at 1 MHz, 1.5 Watts/cm^2, for 10 minutes with a moving transducer using either a direct (gel) or an indirect (underwater) contact technique in tap water with 1 cm distance between transducer and skin. Increases in tissue temperature were recorded at 3 cm depth. The mean increase in temperature

was 4.8°C for the direct (gel), and 2.1°C for the indirect (subaqueous) contact method.

Therefore, the direct (gel) contact technique has consistently been demonstrated to be more effective than the indirect (subaqueous) contact technique and should remain the technique of choice for all ultrasound applications.

b. Angle of application of the transducer.

The application of ultrasound to various parts of the body would imply that the flat surface of ultrasonic transducer would have to be in contact with different body contours, specifically a flat surface or a rounded surface. The transmission of ultrasound energy through these surfaces is subjected to the natural laws of physics and will be reflected and refracted according to the incident angle (Williams 1987). Theoretically, a perpendicular (90°) incident angle would allow most of the energy to pass through with minimal reflection, and a decrease in the incident angle would correspond to an increase in reflection of the energy. With flat surfaces, it is always possible to maintain the incident angle at 90°, but with rounded body contours, this may not always be possible, resulting in less efficient heating. Kimura et al (1998) challenged this assumption by investigating the effect of varying the angle of application of the transducer (90°, 80°, 70°, 60°) on tissue heating. A phantom tissue model consisting of agar spheres, ranging in diameter from 0.5 to 3.0 mm, and suspended in distilled water and 10% propanol solution, was used to emulate human tissue. The phantom tissue was exposed to ultrasound (1 MHz, 2.0 Watts/cm^2, 5 minutes) at varying angles (90°, 80°, 70° and 60°) while the increase in temperatures were recorded at 1 minute intervals. Results of this study demonstrated that thermal effects were greatest at 80° and 90° angles of application. This study highlights the importance of maintaining a perpendicular incident angle between the flat surface of

the transducer and the target tissue in order to achieve consistency in treatment applications to body contours that are not naturally flat.

c. Temperature of the coupling agent:

The temperature of the coupling agent may also have an influence on the heating of tissues exposed to ultrasound. It has been demonstrated that mineral oil at 21°C was more effective at heating deeper structures than mineral oil at 24°C, which provided more superficial heating (Lehmann et al 1966a). It is possible that the viscosity (or density) of the mineral oil is dependent upon its temperature; the higher the temperature of the oil, the less viscous (dense) it becomes. Acoustic impedance is inversely related to transmissivity of ultrasound, and is measured by multiplying the density of the material by the speed at which sound travels through it (Williams 1987). Hence, if the density (or viscosity) of the coupling agent changes, so would its acoustic impedance, and also its transmissivity. Changes in transmissivity of the coupling agent would have an influence on the heating of the tissues, as the total energy transmitted can be significantly different. While the viscosity of coupling agents such as mineral oil can be easily altered by varying the temperature, the same cannot be assumed of other coupling agents such as gel, commonly used for direct contact applications.

Oshikoya et al (2000) investigated the effect of varying the temperature of a coupling agent such as a water-based gel, on the intramuscular temperature. The gastrocnemius muscles of 18 healthy subjects were exposed to ultrasound (1 MHz, 1.5 Watts/cm^2) using a water-based coupling gel at temperatures of 18°C (cooled), 25°C (room temperature), and 39°C (heated). Increases in tissue temperature were recorded at 5 cm depth at 30-second intervals until the temperature reached 4°C above baseline. The rate of intramuscular temperature rise (RTR) was calculated and analyzed. Results showed that the RTR was significantly faster using the 25°C

gel compared with the 18°C and 39°C gels. There was no difference in the RTR between the 18°C and 39°C gel. Oshikoya et al (2000) concluded that the use of a cooled or heated gel might be counterproductive when maximal thermal effects are desired, and that coupling gel at room temperature was preferable to both cooled and heated gel.

d. Pressure of the transducer on the skin:

When moving the transducer over the treatment area, a certain amount of pressure is needed to maintain contact between the skin and the transducer. Because of the elastic nature of skin and soft-tissues, any application of force or pressure deforms the contours of the tissue and may possibly affect the transmissivity of the tissue to the ultrasound energy.

Klucinec et al (1997) investigated the effects of the transducer pressure on acoustic transmissivity. Pig tissues were exposed to ultrasound (1 MHz, 0.5 Watts/cm^2). Pressure on the transducer was maintained by weights at 100g increments from 200 to 1,400 grams. An ultrasound receiver recorded the energy that was being transmitted through the tissues as the pressure on the transducer was varied. The results demonstrated that increased pressure on the transducer had an effect on acoustic transmissivity. Acoustic energy transmission was increased from 200 grams up to and optimally at 600 grams. However, there was decreased transmissivity from 700 to 1,400 grams. A typical transducer would weigh between 300 grams to 500 grams. To maintain consistency in the pressure applied to the transducer during an ultrasonic application, the weight of the transducer head itself may be sufficient to maintain contact with the skin and at the same time provide optimum transmissivity for the acoustic energy.

e. Size of the treatment area:

The size of the treatment area in a therapeutic ultrasound application usually varies with the type of application, pathology and anatomical site. The size of treatment area is usually measured in multiples of the size of the transducer or the effective radiating area (ERA). On average, most treatment applications involve at least 2 X ERA size, but some applications (such as over the shoulder or back) can cover as much as 4 to 8 X ERA. The effect of varying the size of the treatment area on temperature changes in the human patellar tendon was investigated by Chan et al (1998). The patellar tendons of 8 healthy subjects were exposed to ultrasound (3 MHz, 1 Watt/cm^2, 4 minutes) for two different treatment sizes corresponding to twice and four times the ERA of the transducer. Increases in tissue temperature were recorded at 1 cm depth. Results showed that the mean increase in temperature were significantly different for the two treatment sizes (8.3°C for the 2XERA, and 5.0°C for the 4XERA). Chan et al (1998) concluded that the smaller treatment size (2XERA) was more effective, providing higher and longer heating than the 4XERA treatment size.

This study (Chan et al 1998) was the only one found that investigated the effect of varying the size of treatment area on amount of heating in the subcutaneous tissues. However, only one frequency (3 MHz) was investigated. It is assumed that the same relationship exists for 1 MHz but this is yet to be confirmed. Nevertheless, the results of this study (Chan et al 1998) highlight the importance of standardizing the size of the treatment area to maintain consistency in treatment applications and to enable dosages among different studies to be compared.

f. Speed of moving the transducer:

In general, a moving transducer technique should be used to avoid standing wave formation. Standing waves may give rise to slowing or arresting of blood cell flow, transient cavitation and increased microstreaming (Dyson 1987).

In the "Guide Lines for the Safe Use of Ultrasound Therapy Equipment" published by the Chartered Society of Physiotherapy (1990), it is stated that "the transducer should be moved slowly and continuously throughout treatment", possibly to reduce the sensation of hot spots under the transducer, as well as the formation of standing waves (Low and Reed 2000). Hence, therapists usually move the transducer in either a circular fashion or a linear stroking manner, at a fixed or variable rate. There have been no studies to date that examined the effect of varying the speed of movement of the transducer on the rate of heating. It is possible that varying the movement speed of the transducer would have an influence on the rate of heating observed in the tissues. However, without further investigation, it is not possible to make any definite conclusions at this time.

g. Summary

During a therapeutic ultrasound treatment session, the protocol-related factors that can be considered important for the consistency of delivering the same dose of ultrasonic energy to the target tissues are technique of application (direct versus indirect contact techniques), angle of application of the transducer, pressure of the transducer on the skin, the size of the treatment area and the speed of moving the transducer. For most applications, the direct in-contact technique is considered to be most consistent. The angle of the transducer should be perpendicular to the surface of the skin, with the weight of the transducer as the only pressure applied on the skin's surface. For 3 MHz ultrasonic devices, the size of the treatment area

should be kept small (2X ERA), although it is unclear if this is also true for 1 MHz devices. The optimum speed for moving the transducer is unknown.

2.5 RELATIONSHIP BETWEEN FREQUENCY, INTENSITY AND DURATION OF EXPOSURE ON THE HEATING PATTERN

From the preceding section (section 2.3) it appears that to select an appropriate treatment dosage based on the desired increase in target tissue temperature, the relationship between frequency, intensity and duration of exposure on the heating pattern needs to be clarified. While some of the information is available, most of the studies have not set out with the intention to clarify this relationship as their primary objective (Draper and Sunderland 1993, Draper et al 1993, 1995b, 1998a, Rimington 1994, Myrer et al 2001). Essentially, all of the studies reported by Draper and Sunderland (1993), and Draper et al (1993, 1995b, 1998a), used human subjects and measured the *in vivo* temperature of the gastrocnemius muscle at various depths during exposure to ultrasound for 10 minutes at various frequencies and intensities, and under different conditions. Some other studies have looked specifically at the relationship between frequency, intensity and duration of exposure on depth of heating (Draper et al 1995a, Levine et al 2001, Cambier et al 2001), but their methods varied. Draper et al (1995a) used an *in vivo* human model, while Levine et al (2001) used an *in vivo* dog model, and Cambier et al (2001) used a human cadaveric model. Both Draper et al (1995a) and Cambier et al (2001) investigated the 1MHz and 3MHz frequencies, while Levine et al (2001) investigated the 3.3MHz ultrasonic frequency. In addition, while Draper et al (1995a) and Levine et al (2001) used the moving transducer technique, Cambier et al (2001) chose the stationary transducer technique. None of the researchers reported

Table 2.1: Increase in subcutaneous temperature after 10 minutes exposure to 1 MHz ultrasound at various intensities (size of treatment area 2X ERA)

		Increase in temperature (°C) after 10 minutes exposure to ultrasound at corresponding depth of tissue from the surface (in cm)						
Authors	Intensity	1.0 cm	1.5 cm	2.0 cm	2.5 cm	3.0 cm	4.0 cm	5.0 cm
Draper et al 1995a	0.5				0.40*			0.60*
Draper et al 1995a	1.0				1.60*			1.60
Draper and Sunderland 1993	1.5					4.90		
Draper et al 1993	1.5					4.80		
Draper et al 1995b	1.5							4.00
Draper et al 1998a	1.5	3.50				3.85		
Draper et al 1995a	1.5				3.30*			3.30*
Rimington 1994	1.5					2		
Draper et al 1995a	2.0				4.00*			3.40*

*based on an estimate of the reported rate of increase per minute

Table 2.2: Increase in subcutaneous temperature after 10 minutes exposure to 3 MHz ultrasound at various intensities (size of treatment area 2X ERA)

		Increase in temperature (°C) after 10 minutes exposure to ultrasound at corresponding depth of tissue from the surface (in cm)						
Authors	Intensity	1.0 cm	1.5 cm	2.0 cm	2.5 cm	3.0 cm	4.0 cm	5.0 cm
Draper et al 1995a	0.5	3.00*	3.00*					
Draper et al 1995a	1.0	5.80*	5.80*					
Levine et al 2001**	1.0	3.00		2.30		1.60		
Myrer et al 2001	1.0	7.47						
Draper et al 1995a	1.5	8.90*	8.90*					
Levine et al 2001**	1.5	4.60		3.60		2.40		
Draper et al 1995a	2.0	1.50	1.30					

* based on an estimate of the reported rate of increase per minute; ** 3.3 MHz frequency

on the reliability and reproducibility of their procedures. Hence, comparison was difficult across the studies. The results of these studies are summarized in Tables 2.1 (for 1 MHz) and 2.2 (for 3 MHz). As is evident from Tables 2.1 and 2.2, these studies collectively do not provide sufficient details to formulate any useful guidelines for determining treatment dosages.

While the information from these studies has helped in understanding the extent of heating that occurs within the tissues, it is obvious that the information available is not comprehensive. This situation is largely due to the limitations of the studies and the choice of an appropriate animal model and measurement techniques.

While *in vivo* experiments are important to examine the effect of circulation on the heating pattern, they also have some major disadvantages. *In vivo* experiments do not allow the investigator access to the full range of heating that occurs within the tissues. In order to appreciate both the quantitative (increase in temperature) and qualitative (distribution of the heating pattern) aspects of heating in the tissues exposed to ultrasound, and how this is affected by varying the dosage parameters of ultrasound, a cross-sectional view of the heating pattern is necessary to provide full access to the various depths of tissue at which heating occurs.

Furthermore, the measurement of tissue temperature with thermistor probes records the changes in temperature only at specific point locations. While this is useful to record changes in temperature at specific tissue depths, it does not provide for measurement of "area" temperature changes. This "area" measurement of temperature is essential to determine the entire heating pattern that occurs with exposure to ultrasound. Current technology offers several options to measure point as well as area temperatures in tissues. One of the most common methods used in current research is infra-red thermography (Armstrong et al 1997). Infra-red

thermography units have sensitivity up to 0.01°C and can provide both point and area measurement of temperatures. Their non-contact application has both advantages and disadvantages. Since they are non-invasive, they would not be suitable for *in vivo* models if access to the cross-sectional heating pattern were desired. Hence, the effect of blood circulation on the heating pattern cannot be accounted for with this type of instrumentation. However, the common criticism regarding in-situ probes in *in vivo* studies is that the probes themselves can become heated when exposed to ultrasound and therefore cause unknown measurement artifacts (Lehmann 1982). Non-contact temperature sensors such as infra-red thermography do not have to contend with this problem. The validity of infra-red video thermography and infra-red spot (beam) thermography had been investigated by Sherman et al (1996). Comparisons were made between the two recording instruments using a heat-producing reference device. Sherman et al (1996) reported a Spearman's correlation coefficient of 0.994 for the infra-red video thermography and 0.990 for the infra-red spot (beam) thermography. They concluded that both infra-red video and spot thermography devices were highly valid and accurate instruments for measurement of temperatures.

The choice of a suitable animal model is also critical to overcoming some of the limitations of previous studies. There have been several studies investigating the effect of varying the frequency, intensity or duration of exposure on the rate of heating in different animal tissues such as rats (Martin et al, 1984), dogs (Hynynen 1987, Moros and Hynynen 1992, Levine et al 2001), pigs (Lehmann et al 1967b, Lehmann et al 1968, Gammell et al 1979,Goss et al 1979, ter Haar and Hopewell 1985, Ward and Robertson 1996), and humans (Lehmann et al 1967a, Kramer 1984, Draper and Sunderland 1993, Rimington et al 1994, Draper et al 1995a, 1995b, Chan et al 1998, Draper et al 1998a, 1998b, Zemke et al 1998, Wu et al 2000, Cambier et

al 2001, Myrer et al 2001). While human tissue provides the ideal model, it also has several disadvantages. Firstly, *in vivo* studies that allow full access to the cross-section of the heating pattern cannot be carried out. Human cadaveric studies do provide an alternative, but availability of cadavers is a major limitation. An alternative is to find an animal model that approximates closely the human model in terms of tissue composition and size. The pig model (Lehmann et al 1967a, Lehmann et al 1968, Gammell et al 1979, Goss et al 1979, ter Haar and Hopewell 1985, Ward and Robertson 1996), has been used in similar types of studies and, according to Lavker et al (1991), meets all the requirements of being similar in size to humans as well as having similar tissue (fat, muscle and bone) composition. The choice of a pig model, using infra-red thermography to access the cross-sectional heating pattern of both point and area changes in temperatures seems to be a viable model to examine the relationship between frequency, intensity, and duration of exposure to ultrasound and tissue heating at various target depths. Data from such investigations can be sufficiently comprehensive to allow the formulation of a model or treatment guidelines to assist physiotherapists in making an appropriate decision on treatment dosage.

2.6 REVIEWING THE BIOPHYSICAL AND CLINICAL EVIDENCE

With any review of the literature, and therapeutic ultrasound is no exception, it is difficult to make sense of the huge volume of published information without an overall perspective and appreciation of the historical, social and professional developments that influenced the research culture and activities related to therapeutic ultrasound specifically, and the use of electrophysical agents in general. A review of these issues, prior to examining each of the individual studies, is presented in the following sections.

2.6.1 The Research Model for Electrophysical Agents

A number of the electrophysical agents currently in use have been available for many years (Low and Reed 2000). At the time they were developed, the possibility of application to patients was supported by no more than biological plausibility. For most of these modalities, there was little or no preliminary animal testing, and even clinical trials were mostly pre-empted by widespread application in the clinical setting. In view of the long-standing tradition of clinical usage, preliminary experiments investigating the basic effects of these modalities have, in many instances, continued to be neglected. The lack of background information about the biophysical effects of these modalities hampers the design and interpretation of ongoing clinical studies. Hence, it would be of value to take a step back and adopt a planned research approach, such as that widely utilized in contemporary pharmaceutical research to examine efficacy of new drug interventions.

The pharmaceutical research model for investigating and testing new drugs begins with either the discovery of new chemical compounds or an accidental

discovery that a chemical compound being tested for its effect on an identified disease exhibits potential in the treatment of other unrelated diseases (Basmajian 1991). This discovery usually initiates a chain of events (summarized in Figure 2.1), beginning with initial testing in animals, to document the toxicity, metabolism, excretion and other biological effects of the new drug. It is not unusual for a pharmaceutical company to have accumulated 2 to 5 years of data on animals before testing on human subjects is approved (Basmajian 1991).

Testing on human subjects is carried out in three phases (Basmajian 1991). Phase 1 human studies are carried out under the supervision of expert investigators. During this phase, the drug is administered to only a few closely monitored normal individuals with a view to determining its safety rather than its efficacy.

Phase 2 involves administration of the drug to a larger group, approximately 200 patients, to examine the efficacy of the drug. Safety studies may also be repeated, but they are not the primary purpose of this phase.

Phase 3 involves field trials in which the drug is administered to a larger group of patients, under conditions approaching general clinical practice. The purpose of Phase 3 trials is to determine the effectiveness of the drug intervention. Phase 3 studies are often very large and require involvement of a number of different centres to access sufficient volunteers in an appropriate time-frame.

Phase 2 and 3 trials are most often undertaken using randomized controlled trials (RCTs). RCTs are ideal for evaluating the effectiveness of various forms of treatment interventions. Patients are randomly assigned to an experimental (or treatment) group, a control (or non-treatment) group, and sometimes, a placebo group. To evaluate the effectiveness of the drug treatment, group comparisons are made (Basmajian 1991).

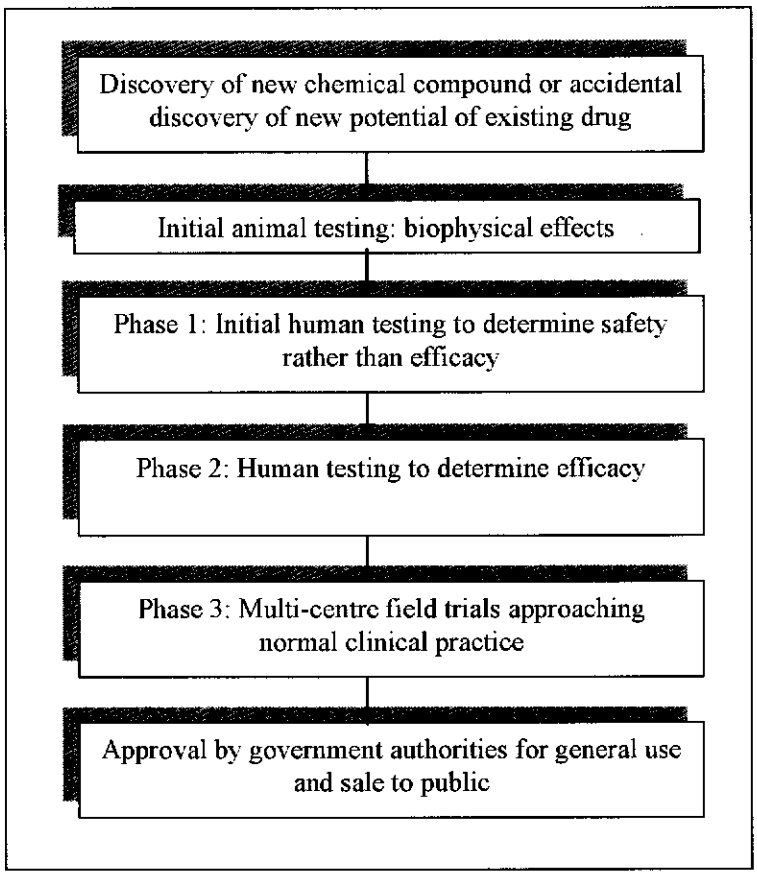

Figure 2.1: Summary of Pharmaceutical Research Model

A modification of this pharmaceutical model for adoption in EPA research is proposed in Figure 2.2. This proposed model would facilitate identification of gaps in physiotherapy research to date. This would discourage implementation of Phase 2 or Phase 3 clinical trials with larger samples prior to appropriate preliminary *in vitro* and *in vivo* animal (and cellular) studies (Laakso et al 2002). International adoption of a credible research model for electrophysical agents, such as that proposed in Figure 2.2, would expedite efforts to provide evidence for the modalities already used in clinical practice on a daily basis.

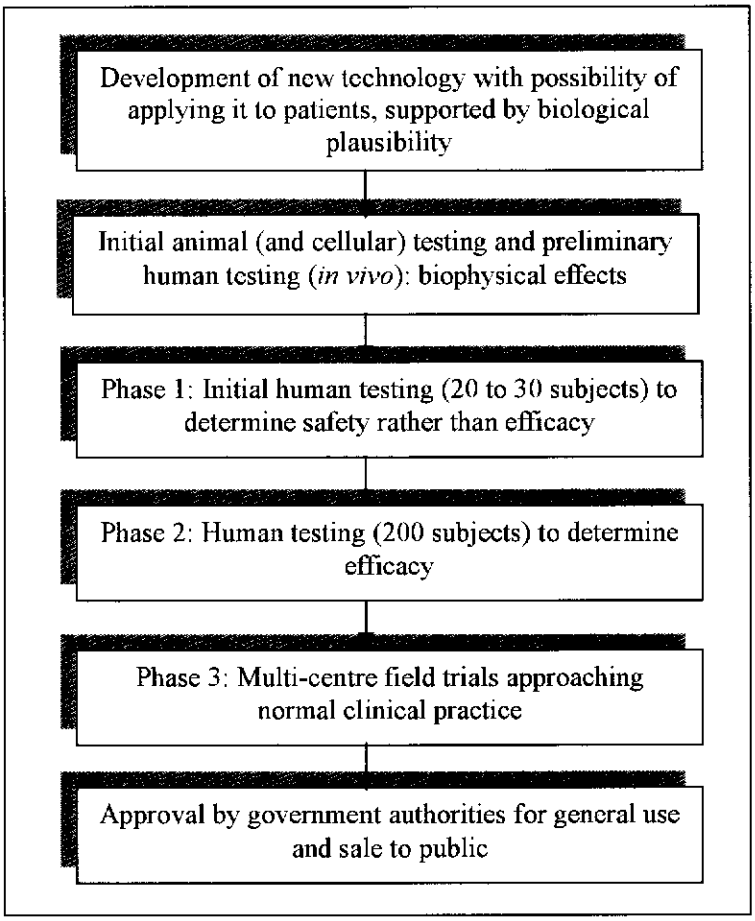

Figure 2.2: Summary of Proposed EPA Research Model

2.6.2 The Status of Research in Therapeutic Ultrasound

In order to systematically examine all the research related to therapeutic ultrasound and evaluate it within the context of a possible research model (Figure 2.2), a literature review was conducted. Sources used were *PubMed* and *Cinahl Direct*. *PubMed* (http://www.ncbi.nlm.nih.gov/PubMed/) was used to access the MEDLINE database from 1966 to December 2001. *Cinahl Direct* (http://www.cinahl.com) was used to access the CINAHL database from 1982 to December 2001.

The same keywords ("ultrasonic therapy") were used in the search strategies for both the MEDLINE and CINAHL databases and this yielded 3993 and 229 hits respectively. Each of these lists was manually screened to select only articles that were related specifically to physiotherapy. Not counting duplicates as well as inappropriate references, the total number of relevant articles found through the manual screening process was 209. These articles were categorized according to the type of research such as equipment performance, reviews, surveys, initial testing on animals and humans, and Phase 1, Phase 2 and Phase 3 clinical studies (Figure 2.3).

In addition, the distribution of research activities through the various decades was identified (Figure 2.4). The types of tissue specimens selected for the initial testing phases (Figure 2.5), and the distribution of this work reported through the decades (Figure 2.6) were also summarized.

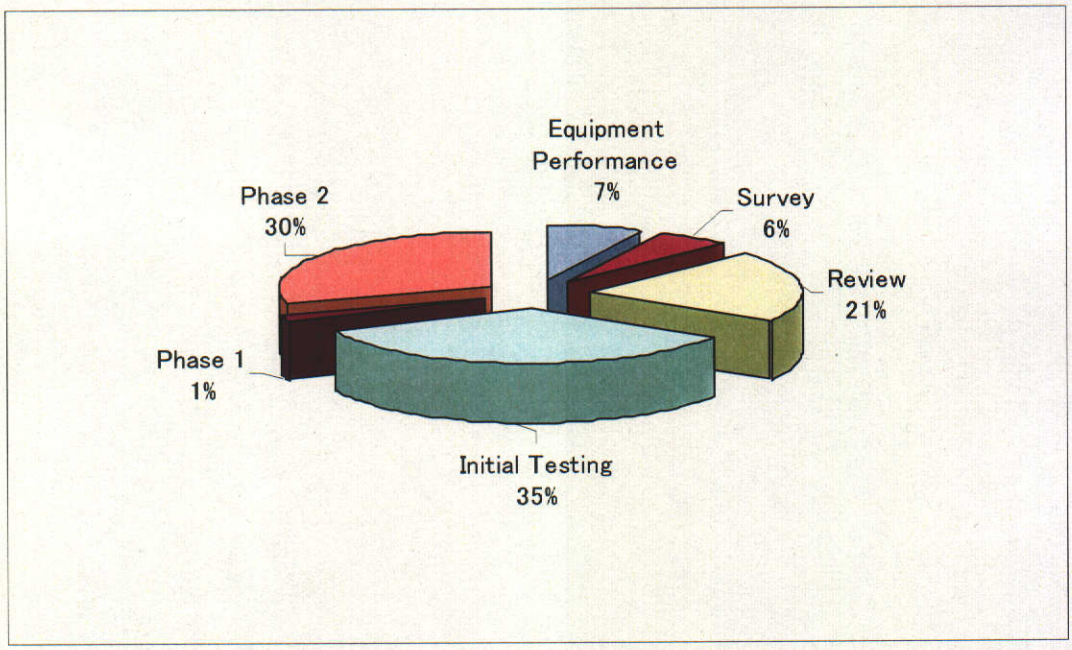

Figure 2.3: Types of Research Activities in therapeutic ultrasound (n=209)

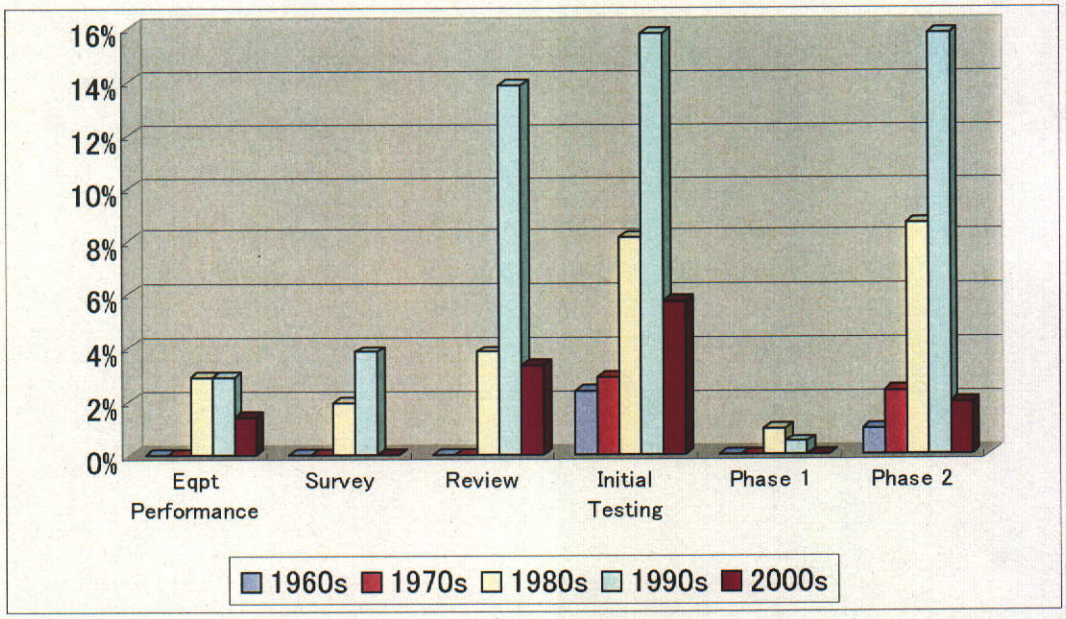

Figure 2.4: Distribution of the various types of research activities in therapeutic ultrasound through the decades (n=209)

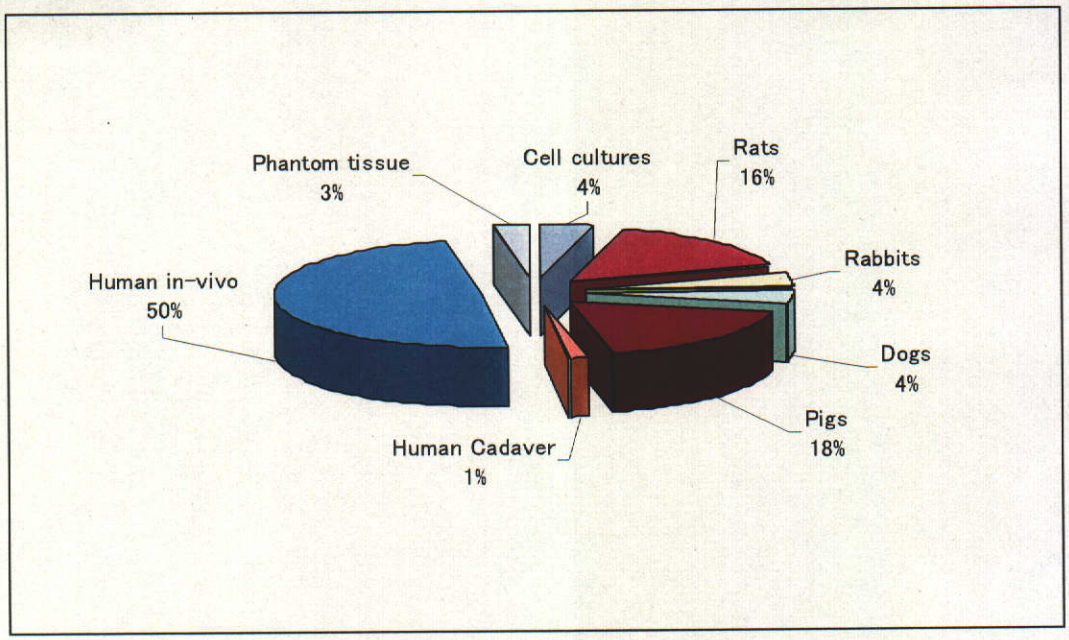

Figure 2.5: Types of tissue used for initial animal testing in therapeutic ultrasound (n=73)

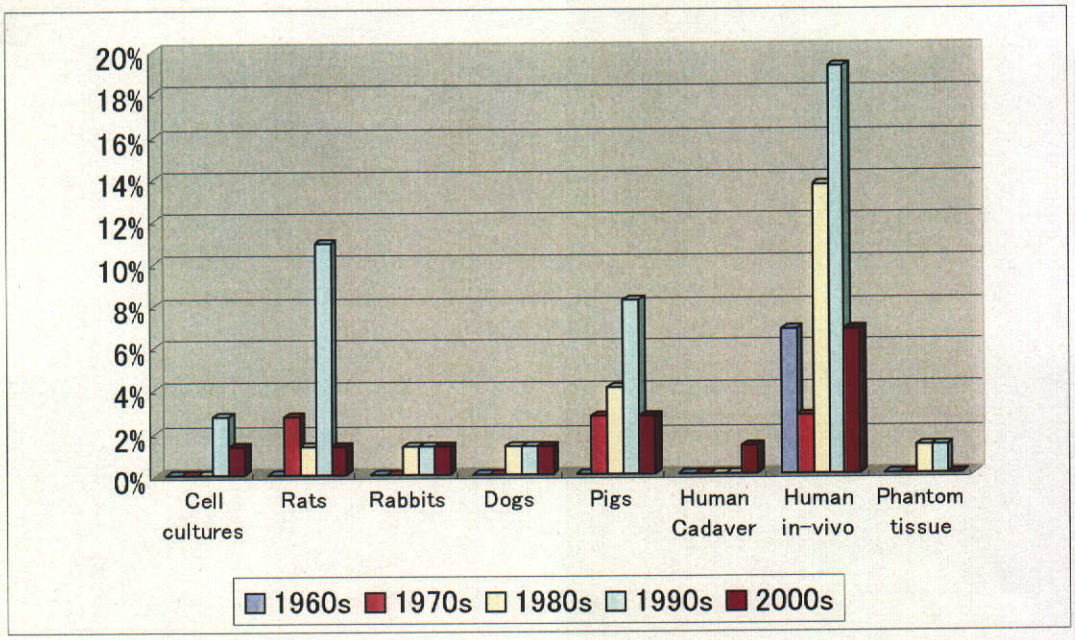

Figure 2.6: Distribution of the types of tissue used for research in therapeutic ultrasound through the decades (n=73)

From the information summarized in Figures 2.3 to 2.6 (pp46-47), some interesting observations need to be highlighted:

a. Using the proposed electrophysical agents (EPA) research model as a template for comparison (Figure 2.2), it can be seen from Figures 2.3 and 2.4 that there are gaps in the reported research activities over the last four decades. While initial testing and Phase 2 studies accounted for about 35% and 30% respectively of all published studies to date, Phase 1 (1%) and Phase 3 (0%) studies were noticeably lacking. Despite this, critical reviews (6%) and meta-analyses (1%) are being published every year based on studies that are flawed, poorly conducted, or generally incomplete. These reviews also regularly pass judgment on the effectiveness of the modality despite the inappropriate nature of the studies examined.

b. Even though the percentage of initial studies (35%) and phase 2 studies (30%) appeared to be acceptable (Figure 2.3), on closer examination, it can be seen that both initial testing and phase 2 studies were being conducted concurrently (Figure 2.4). Ideally, based on the proposed EPA research model, initial testing should precede phase 2 studies, as the information obtained from initial animal testing is a pre-requisite to successful implementation of phase 2 studies. Information from initial animal testing would include answers to dosimetry issues, protocol-related issues, and appropriate selection of patient populations most likely to benefit from ultrasound treatment. When both initial studies and phase 2 studies are being conducted concurrently, at least some of the phase 2 studies may include unnecessary design weaknesses that may limit the interpretation of their results.

c. From Figure 2.3, it can be seen that while phase 2 studies constituted about

30% of research activities, only 18% were of appropriate design (i.e. RCTs). The other 12% were non-RCTs and therefore, had to be rejected, as they did not meet the minimum standard for determining effectiveness of the modality. Of the 18% RCTs, almost all had some methodological flaws which will be further elaborated upon in Section 2.6.3 (p50). It can be suggested that almost all the phase 2 studies that had been conducted to date were of limited value as they were unable to identify the effectiveness of the modality.

d. Initial animal testing accounted for 35% of all the ultrasound research identified in this review (Figure 2.3). However, there was no evidence of a logical progression from *in vitro* cell culture investigations, to *in vitro and in vivo* small animal studies and finally to *in vitro* and *in vivo* large animal studies. The studies identified (Figure 2.6) suggested that all three types of investigations had been proceeding concurrently. Furthermore, while the choice of rats (16%), rabbits (4%) and dogs (4%) for small animal studies, and the choice of pigs (18%) for large animal studies, may be considered appropriate, it was unclear why so many studies using human subjects (50%) had been undertaken prior to analogous animal studies. While the potential dangers associated with therapeutic ultrasound are far less than many other therapeutic interventions, damage and harm to experimental subjects cannot be totally excluded. For this reason, it would be prudent to postpone human trials until after preliminary animal studies have been completed. This approach is likely to reduce the numbers of human volunteers ultimately required and has obvious ethical and methodological advantages.

Summary

Adopting a more coherent and rational research model, as proposed in Figure 2.2 would appear to have much to offer in improving the current status of research in therapeutic ultrasound.

2.6.3 Biophysical Effects and Clinical Effectiveness of Therapeutic Ultrasound: From *in vitro* to *in vivo* to RCTs

During the 1920s and 1930s, much was learnt about the effects of directing ultrasonic energy through biological tissues (Nyborg, 2001). Wood and Loomis (1927; cited in Fyfe & Bullock 1985) have been credited with being the first investigators to examine the biophysical effects of therapeutic ultrasound during this early period (Fyfe & Bullock 1985; ter Haar 1999; Nyborg 2001). By using a specially constructed ultrasonic generator capable of producing an output estimated at 10 W/cm^2, they were able to demonstrate the death and destruction of small fish and frogs exposed to this ultrasonic energy (Wood & Loomis 1927, cited in Fyfe & Bullock 1985). In addition, duration of exposures of one to two minutes caused the destruction of red blood cells and temporary immobilization of unicellular organisms such as paramecium (Wood & Loomis 1927; cited in Fyfe & Bullock 1985).

In 1930, Harvey reviewed the evidence on the biophysical effects of ultrasound uncovered at that time. He was able to identify three mechanisms that appeared to be implicated in the production of the biophysical effects. They were: heating of the medium from absorption of the ultrasonic energy; movement and aggregation of particles from acoustic streaming and cavitation activity (Harvey 1930, cited in Nyborg 2001). The first mechanism is commonly known as "thermal effects", and the second and third mechanisms are collectively referred to as "non-thermal effects". While these three known mechanisms of ultrasound were

considered true and remained unchallenged today, attributing any observed biophysical effects to each or any of these mechanisms remains controversial (Fyfe & Bullock 1985; ter Haar 1999; Nyborg 2001). In fact, ter Haar (1999) cautioned that:

> "... it is ... often extremely difficult to identify positively the mechanisms involved in producing biological change, and indeed to isolate non-thermal effects from thermal ones" (ter Haar 1999, p. 4).

Consequently, the clinical applications of ultrasound have not always developed from the demonstrated biophysical effects. Often, clinical applications originated from assumed biological plausibility, without confirmation of the postulated biophysical effects in either *in vitro* or *in vivo* animal studies.

The first reported clinical application of therapeutic ultrasound was in Germany in 1938 (Nyborg 2001). In the reported case study, the patient was diagnosed as having sciatica. Therapeutic ultrasound at a frequency of 800 kHz and an intensity of 4 to 5 W/cm^2 was applied to the patient. Following this, therapeutic ultrasound was used to treat hundreds of patients with sciatica, neuralgia and other similar conditions in Europe and North America (Nyborg 2001). Today, therapeutic ultrasound is used for the treatment of various conditions and disorders such as soft tissue injuries, tissue healing, obstetric conditions, scar tissue, dental conditions, and for pain relief, among others (McDiarmid and Burns 1987, Low and Reed 2000).

Biophysical effects of ultrasound from *in vitro* and *in vivo* animal and cellular studies and the clinical effectiveness of the modality based on RCTs represent different phases of the investigative continuum (Laakso et al 2002), with the *in vitro* and *in vivo* animal and cellular studies preceding the clinical studies (see Figure 2.2). Based on the summary characteristics of the literature survey described in Section 2.6.2 (pp 45-50), therapeutic use of ultrasound can be divided into four

categories. These correspond to the most common clinical applications of ultrasound in patient populations and include:

a. Control of inflammation

b. Control of pain

c. Facilitation of tissue healing

d. Changing tissue extensibility

As might be expected, there may be some overlap between categories as some clinical applications aim to produce more than one biophysical effect. For the purpose of the following review, papers have been categorized according to their intended main effect. In addition, the papers cited have been grouped within each category according to whether they represent initial animal studies, phase 1 or phase 2 studies. No phase 3 studies were identified.

a. Control of inflammation

The inflammatory process is the first stage in the healing of soft tissues. It has been suggested that inflammation usually begins within a time frame of "soon after injury" to about 12 hours post injury, and continues for 3 to 7 days, depending on the severity of insult to the tissues (Dyson 1987, Birkett 1999). The four cardinal signs and *sequelae* of inflammation are *rubor* (redness), *calor* (heat), *tumor* (swelling), and *dolor* (pain), with subsequent loss of function (Birkett 1999; Figure 2.7).

Acute inflammation is characterized by an increase in white blood cells, as well as polymorphonuclear leucocytes (PMNs). The series of events in the process of acute inflammation had been described by Birkett (1999) as follows (see Figure 2.7):

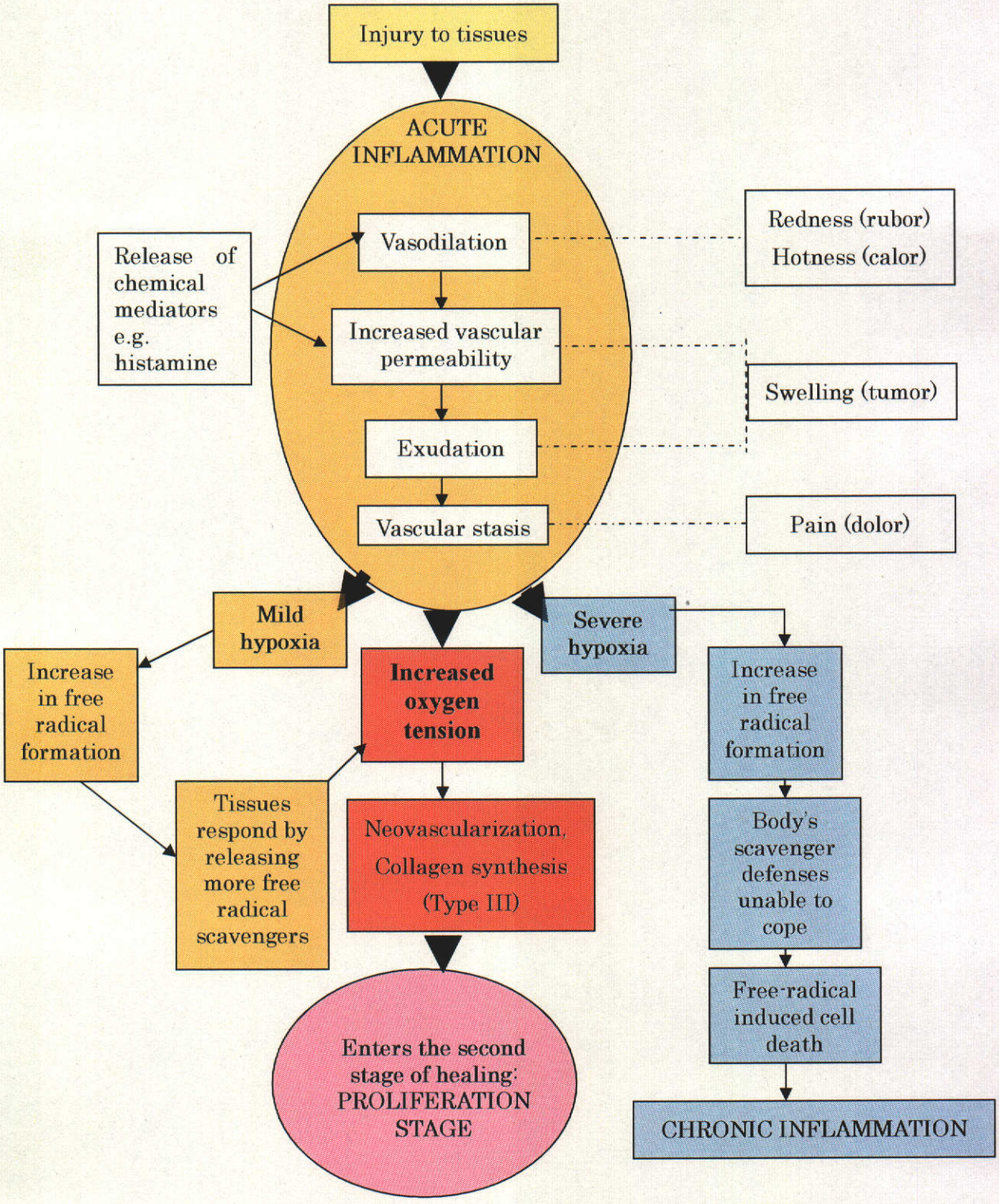

Figure 2.7: Inflammation – The first stage in the healing process

- Vasodilation. The initial but transient vasoconstriction of arterioles is mediated by both neurogenic and vasoactive agents, and is rapidly followed by arteriolar vasodilation. The pre-capillary sphincters open, leading to all the capillaries in the microvascular bed being perfused. This increase in blood flow to the area of inflammation results in the first and second cardinal signs of inflammation, redness (*rubor*) and heat (*calor*).
- Increased vascular permeability. In the post-capillary venules, vasoactive mediators begin to bind to endothelial cells, causing contraction of micro-filaments and intercellular separation, hence allowing extravasation of fluid into the extravascular space.
- Exudation. As a result of increased osmotic pressure extravascularly and increased hydrostatic pressure intravascularly, there is an escape of fluid, proteins, red blood cells and white blood cells from the intravascular space, eliciting the third cardinal sign of inflammation, swelling (*tumor*).
- Vascular stasis. The net effect of vasodilation and fluid exudation is a slowing down of blood in the bloodstream, or vascular stasis. The vascular stasis can allow chemical mediators and inflammatory cells to collect and respond to this stimulus, thereby eliciting the fourth cardinal sign of inflammation, pain (*dolor*).

The acute inflammatory process occurs in a tissue environment of decreased oxygen tension (Maxwell 1992). Chemical mediators, such as histamine, are released, causing vasodilation and increased capillary permeability. At the same time, these chemical mediators also attract macrophages that are responsible for the removal of tissue debris and pathogens from the injured sites (Maxwell 1992, Birkett 1999). These chemical mediators, such as cytokines, also cause the migration of fibroblasts and endothelial cells into the area. After five days, the tissue environment demonstrates an increase in oxygen tension, and this coincides with

neovascularization and collagen synthesis, both of which are dependent upon oxygen. In this scenario, the acute inflammatory process and the cascade of events leading up to neovascularization and collagen synthesis, enters the second phase of healing known as the proliferation stage (Maxwell 1992, Birkett 1999). The tissue environment can also become hypoxic (Maxwell 1992), and this can have significant impact on the healing process. In a hypoxic tissue environment, an increase in free radical formation occurs (Maxwell 1992). Free radicals are chemicals that are produced normally in cells, and are usually removed by free radical scavengers. In a mildly hypoxic environment, the free radical scavengers are able to remove the excess free radicals (Halliwell 1994). However, in severe hypoxia, the free radical scavengers are unable to cope, causing this defense mechanism to break down, leading to tissue destruction and cell death induced by the free radicals. Halliwell (1994) suggests that the damage caused by these free radicals, under severe hypoxic conditions, plays a significant role in the development of chronic inflammation.

It is widely believed that inflammation is necessary for tissue healing to occur (Maxwell 1992, Birkett 1999). However, excessive inflammation can lead to swelling, pain, scar formation, secondary tissue ischaemia, loss of motion and weakness (Birkett 1999). It has been suggested that therapeutic ultrasound at low intensities can accelerate the resolution of the inflammatory response (anti-inflammatory effect) but at higher intensities, therapeutic ultrasound can promote excessive inflammation which can be even more detrimental to the injured tissues (pro-inflammatory effect) (Dyson 1987). The key to successful treatment outcomes lies in applying the "most appropriate dosage at the most effective stage of the healing process" (Maxwell 1992, p424). Exactly what is considered an appropriate dose, however, is unclear.

Table 2.3: Summary of initial studies investigating the effect of ultrasound on inflammation

Author	Type of study	Frequency / Duration	Intensity (W/cm^2)	Effect on Inflammation
Pro-Inflammatory effects				
Hogan et al 1982a	Muscle (rat *in vivo*)	1 MHz / 5 minutes	Pulsed, SATP 1.25, 2.5, 5, 10	No change in blood flow at 1.25 and 2.5; Decrease in blood flow at 5 and 10
Hogan et al 1982b	Muscle (rat *in vivo*)	1 MHz / 5 minutes	Pulsed, SATP 1.25, 2.5, 5, 10	No change in blood flow at 1.25 and 2.5; Decrease in blood flow at 5 and 10
Klemp et al 1982	Muscle (human *in vivo*)	? MHz / 6 minutes	SATP 1.0	Decrease in blood flow
Rubin et al 1990	Muscle (rat *in vivo*)	1 MHz / 3 minutes	Pulsed, SATP 2.5, 5.0	No change at 2.5; Decrease in blood flow at 5.0
Anti-inflammatory effects				
Bickford & Duff 1953	Muscle (human *in vivo*)	0.8 MHz / 10 to 15 minutes	SATP 2.0, 3 to 3.5	Increase in blood flow
Paul & Imig 1955	Muscle (human *in vivo*)	? MHz / 20 minutes	SATP 2.0, 3 to 3.5	Increase in blood flow
Fyfe & Chahl 1984	Muscle (rat *in vivo*)	0.75, 1.5, 3.0 MHz / 1 minute	Pulsed, SATP 0.5	Increase in vascular perfusion
Young & Dyson 1990	Cell culture (Calf *in vitro*)	0.75 or 3 MHz / 5 minutes	Cont, SATP 0.50.	0.75MHz affected membrane permeability; 3.0MHz increased fibroblasts proliferation
De Deyne & Kirsch-Volders 1995	Cell culture (human *in vitro*)	1 MHz / 0, 30, 60, 90 seconds	Pulsed (20%), SATP 1.0	Facilitation of phagocytosis during chronic inflammation

SATP = Spatial Averaged Temporal Peak; Cont = Continuous

(1) Initial studies

Initial studies investigating the effects of ultrasound on inflammation have been classified as either pro-inflammatory or anti-inflammatory (see Table 2.3). Increase in arterial circulation during exposure to ultrasound (an anti-inflammatory effect) is a well-known effect (Bickford & Duff 1953, Paul & Imig 1955, Fyfe & Chahl 1984). However more recent studies have indicated that in acute inflammation, ultrasound can also cause vasoconstriction of the small arterioles, leading to a local

decrease in circulation (pro-inflammatory effect) (Hogan et al 1982a, 1982b, Klemp et al 1982, Rubin et al 1990). It is possible that the reduction in circulation can lead to local hypoxia or even ischaemia, and under these circumstances, ultrasound treatment in acute inflammation is not only counter-productive, but also potentially harmful. More investigations are needed to clarify the results reported by Hogan et al (1982a, 1982b), Klemp et al (1982) and Rubin et al (1990). Other anti-inflammatory effects of ultrasound that have been demonstrated include increased cell membrane permeability in response to exposure to 0.75MHz ultrasound, increased fibroblast proliferation in response to exposure to 3MHz ultrasound (Dyson 1990) and facilitation of phagocytosis in chronic inflammation in response to exposure to pulsed 1MHz ultrasound (De Deyne and Kirsch-Volders 1995).

(2) Phase 1 and 2 studies

There is no evidence that any of the clinical studies reviewed and summarized in Table 2.4 have considered any pro-inflammatory effects of ultrasound. Moreover, treatment outcomes are likely to be highly dependent upon applying the most appropriate dosage to optimize inflammation control at each stage of the healing process. The difficulty is that there are currently no reliable guidelines to assist researchers or clinicians to determine what is the optimal dose to achieve the desired control of the inflammatory process. Hence, clinical studies should be interpreted with caution regardless of the rigor of other aspects of the methodology.

Table 2.4: Summary of Phase 2 Randomised Controlled Trials investigating the effect of ultrasound on inflammation

Authors	Diagnosis (No. of subjects)	Frequency / Duration	Intensity (W/cm^2)	Outcome Measures	Outcome
Nyanzi et al 1999	Acute ankle sprains (26 Exp vs 25 Pla)	3 MHz / 10 minutes	Pulsed (20%), SATP 0.25, SATA 0.05	Pain, swelling, ROM	No significant difference between groups
Downing & Weinstein 1986	Subacromial bursitis (11 Exp vs 9 Pla)	1 MHz / 6 minutes	Cont, SATP variable (mean 1.2)	Pain, ROM, ADL	No significant difference between groups
Nykanen M 1995	Rotator cuff syndrome (35 Exp vs 37 Pla)	1 MHz / 10 minutes	Pulsed (20%), SATP 1.0, SATA 0.2	Pain, ADL	Both groups improved, but no significant difference between groups
Ebenbichler et al 1999	Calcific tendonitis Shoulder (32 Exp vs 29 Pla)	0.89 MHz / 15 minutes	Pulsed (20%), 2.5, SATA 0.5	Xray changes, 100 point score	Exp group improved significantly
Van der Heijden et al 1999	Shoulder pain (39 Exp vs 33 Pla vs 35 Con)	0.8 MHz / > 2 minutes	Pulsed (20%), SATP variable	Pain, ROM function	No significant difference between groups
Binder et al 1985	Lateral epicondylitis (25 Exp vs 23 Pla)	1 MHz / 5 to 10 minutes	Pulsed (20%), SATP 1.0 to 2.0, SATA 0.2 to 0.4	Pain, weight lifting, grip strength	Exp group improved significantly
Lundeberg et al 1988	Lateral epicondylitis (33 Exp vs 33 Pla vs 33 Con)	1 MHz / 10 minutes	Cont, SATP 1.0,	Pain, strength	Exp group significantly better than Cont group, but no difference between Exp and Pla
Haker & Lundeberg 1991	Lateral epicondylitis (21 Exp vs 22 Pla)	1 MHz / 10 minutes	Pulsed (20%), SATP 1.0, SATA 0.2	Pain, strength	No significant difference between groups
ElHag et al 1985	Molar extraction (33 Exp vs 32 Pla)	3 MHz / 8 minutes	Pulsed (20%), SATP 0.5	Swelling, trismus, plasma cortisol, wound healing	Exp group improved significantly
Hashish et al 1986	Molar extraction (25 Exp vs 25 Pla vs 25 Con)	3 MHz / 5 minutes	Pulsed (20%), SATP 0.1, 0.5, 1.5, SATA 0.02, 0.1, 0.3	Pain, trismus	Exp and Pla groups significantly better than controls.
Hashish et al 1988	Molar extraction (25 Exp vs 25 Pla vs 50 Con)	3 MHz / 5 minutes	Pulsed (20%), SATP 0.1, SATA 0.02	Pain, trismus	Exp and Pla groups significantly better than controls.

Exp = Experimental group; Pla = Placebo group; Con = Control group; SATP = Spatial Averaged Temporal Peak; SATA = Spatial Averaged Temporal Average; Cont = Continuous; ROM = Range of motion; ADL = Activities of Daily Living

The effects of ultrasound on acute ankle sprains, which usually demonstrate the classical signs of acute inflammation: redness, heat, swelling and pain have been investigated (Nyanzi et al 1999). In this placebo controlled (0.00 Watts/cm^2) study the intervention dosage selected was pulsed (20%), 3MHz for 10 minutes at 0.05 Watts/cm^2 (SATA) and the ultrasound machine was calibrated by an independent examiner. There

was no difference between the two groups in any of the outcome measures.

The effects of ultrasound on shoulder pain have also been examined. The conditions investigated include subacromial bursitis (Downing and Weinstein 1986), rotator cuff syndrome (Nykanen 1995) calcific shoulder tendonitis (Ebenbichler et al 1999) and generalized shoulder pain (Van der Heijden et al 1999). All, except the last study, included ultrasound machine checks and calibrations in their study protocols. Only one study (Ebenbichler et al 1999) demonstrated a significant difference between the experimental and placebo groups; however, this study did not employ a control group.

Three studies examined the effects of ultrasound on lateral epicondylitis. Overall, the results were equivocal, with only one study (Binder et al 1985) demonstrating a clear benefit for using ultrasound in this condition. The other two studies demonstrated that ultrasound was no better than placebo ultrasound (Lundeberg et al 1988, Haker and Lundeberg 1991). All three studies reported that their ultrasound machines were checked and calibrated during the period of their studies. The main reason for Binder et al's (1985) success when compared with the other two studies could be in their careful supervision and monitoring of patients to ensure that they had adequate rest during the course of treatment for both the experimental and placebo groups. In the other two studies, normal activities were allowed, and no attention was paid to the amount of rest received by each patient group. This highlights the importance of ensuring a conducive macro-environment that facilitates healing during the treatment session, and that clinical trials investigating the effectiveness of any modality must take into consideration all other details of the patient's

condition.

Three studies have examined the effects of ultrasound on pain and inflammation following surgical extraction of molars (ElHag et al 1985, Hashish et al 1986, 1988). All three studies reported that the ultrasound machines used were checked and calibrated during the study. Two of three studies (ElHag et al 1985, Hashish 1986) demonstrated that ultrasound was effective in reducing pain and inflammation when compared with the placebo or control group, and that lower intensities were more effective than higher intensities (Hashish et al 1986). However, in a more recent study, Hashish et al (1988) compared the effect of placebo ultrasound and mechanical massage on the pain and inflammation. They concluded that although ultrasound therapy was able to reduce pain and inflammation following molar extraction, the mechanism was placebo-mediated and totally unrelated to ultrasound itself.

(3) Summary

The evidence from the clinical trials investigating the effectiveness of ultrasound on inflammation seems to be equivocal. The main problem with each of these studies is the lack of a reasonable guideline to determine the most appropriate treatment dosage that would affect the inflammatory process in the desired manner. This is particularly important as ultrasound has been shown to have pro-inflammatory as well as anti-inflammatory effects (Table 2.3). The main issue therefore, is not whether ultrasound has an effect on inflammation but rather whether ultrasound is able to influence inflammation in such a manner as to facilitate tissue healing. The key to this is dosimetry, and this can only be resolved by conducting more basic cellular and animal studies, rather than clinical trials.

b. Control of pain

There are two instances where the use of ultrasound has been proposed for the relief of pain (Low and Reed 2000). The first instance involves the treatment of inflammatory conditions where pain is secondary to the inflammatory process (see Figure 2.7, page 53). In the second instance, ultrasound is used to treat conditions such as perineal pain, low back pain, post-operative pain and delayed onset muscle soreness, where pain is the primary symptom that is not associated with an ongoing inflammatory process.

The mechanism of pain relief related to control of inflammation is thought to result from an increase in circulation as a consequence of the absorption of heat in the surrounding tissues (Maxwell 1992). This increased blood flow can lead to the removal of the pain mediators, which are by-products of the inflammatory process, thereby inducing pain relief (Imig et al 1954, Maxwell 1992).

Where pain is not secondary to inflammation, the mechanism of pain relief is thought to be due to the direct action of ultrasound on the sensory nerves (Low and Reed 2000). It has been proposed that heat produced by the ultrasound is readily absorbed by nerve tissue that has a high protein content. This results in increased pain threshold due to a slowing of the conduction velocity in sensory nerves (Falconer et al 1990).

(1) Initial studies

Pain relief due to increased circulation has not been conclusively demonstrated. In addition to the studies summarized in Table 2.3 (p56) which show that ultrasound can decrease the circulation (Hogan et al 1982a, 1982b, Klemp et al 1982, Rubin et al 1990), there are others that demonstrate increases in circulation (Bickford and Duff 1953, Paul and Imig 1955, Fyfe and Chahl 1984), and still others that demonstrate no effect of

Table 2.5: Summary of initial studies investigating the effect of ultrasound on circulation

Author	Type of Study	Frequency / Duration	Intensity (W/cm^2)	Effect on Circulation
Hansen et al 1973	Muscle (human *in vivo*)	? MHz / 5 minutes	SATP 1.0	No change in blood flow
Wyper et al 1978	Muscle (human *in vivo*)	1 MHz & 3 MHz / 6 to 13 minutes	Cont, SATP 1.0, 2.0; Pulsed SATP 0.5, 1.0, 2.0, 3.0	No change in blood flow
Clemente et al 1992	Muscle (human *in vivo*)	1 MHz / 5 minutes	Cont, SATP 1.5; Pulsed SATP 1.5	No change in blood flow
Robinson & Buono 1995	Muscle (human *in vivo*)	1 MHz / 5 minutes	Cont, SATP 1.5	No change in blood flow in muscle, but increase blood flow to skin
Fabrizio et al 1996	Muscle (human *in vivo*)	1 MHz & 3 MHz / 5 to 15 minutes	Cont, SATP 1.0, 1.5	No change in blood flow at 3 MHz; Increase in blood flow at 1 MHz

SATP = Spatial Averaged Temporal Peak; Cont = Continuous

Table 2.6: Summary of initial studies investigating the effect of ultrasound on nerve conduction velocity

Author	Type of Study	Frequency / Duration	Intensity (W/cm^2)	Effect on Nerve Conduction Velocity
Madsen & Gersten 1963	Ulnar (motor) (human *in vivo*)	1 MHz / 5 minutes	Cont SATP 0.88 & 1.28 Cont SATP 1.92	Decreased Increased
Farmer 1968	Ulnar (motor) (human *in vivo*)	0.87 MHz / 5 minutes	Cont SATP 0, 1.5, 2.0 Cont SATP 0.5, 3.0	Decreased Increased
Currier & Kramer 1982	Radial (sensory) (human *in vivo*)	1 MHz / 5 minutes	Cont SATP 1.5	Increased
Cosentino et al 1983	Median (sensory) (human *in vivo*)	1 MHz / 10 minutes	Cont SATP 0.5, 1.0, 1.5	Decreased
Kramer 1985	Ulnar (sensory) (human *in vivo*)	0.87 MHz / 5 minutes	Cont SATP 0.5, 1.0, 1.5, 2.0, 2.5	Increased
Kramer 1987	Ulnar (motor + sensory) (human *in vivo*)	0.87 MHz / 5 minutes	Cont SATP 0.5, 1.0, 1.5, 2.0, 2.5	Increased

SATP = Spatial Averaged Temporal Peak; Cont = Continuous

ultrasound on circulation (Hansen et al 1973; Wyper et al 1978; Clemente et al 1992; Robinson and Buono 1995; Fabrizio et al 1996; see Table 2.5).

The conflicting results could be due to the various methods employed to detect changes in circulation. These methods include using venous occlusion plethysmograph (Bickford & Duff 1953, Paul & Imig 1955, Robinson & Buono 1995), a flowmeter (Paul & Imig 1955, Hogan et

al 1982a, 1982b, Rubin et al 1990), 133 Xenon washouts (Hansen et al 1973, Wyper et al 1978, Klemp et al 1982), dye measured spectrophotometer (Fyfe & Chahl 1984), and ultrasound Doppler (Clemente et al 1992). There appear to be systematic differences in outcome depending on the methods used. For example, increases in circulation were reported more frequently when venous occlusion plethysmography and dye measured spectrophotometery were used; decreases in circulation were reported more frequently when a flowmeter was used; and no change in circulation was reported more frequently when 133 Xenon washout and ultrasound Doppler were used. It is not clear whether these patterns are coincidental or a reflection of technical factors associated with the various methodologies. Regardless, the effect of ultrasound on circulation is yet to be determined.

Similarly, changes in nerve conduction velocity in sensory nerves that have been exposed to ultrasound have yet to be clearly demonstrated (see Table 2.6). Experiments on motor and sensory nerves show that ultrasound can alter the conductivity of nervous tissues either way (Madsen and Gertsten 1963, Farmer 1968, Currier & Kramer 1982, Cosentino et al 1983, Kramer 1985, Kramer 1987), as summarized in Table 2.6. Of the six studies, only three reported that calibration of the ultrasonic output intensity was carried out prior to the experiments (Cosentino et al 1983, Kramer 1985, 1987). The mixed results obtained by Madsen and Gertsten (1963) and Farmer (1968) were difficult to explain, as increased and decreased nerve conduction velocities did not appear to follow any discernible dosage pattern. However, since neither study reported whether calibration of the ultrasound machines was carried out during the period of the study, and since calibration of ultrasound machines was not common practice at that

time, it is possible that machine output error may have confounded the results.

Reduction in the conduction velocity of the sensory nerve was not the mechanism for pain relieve in *in vivo* studies of human subjects (Kramer 1985 and 1987). Calibration of the ultrasound machines used in these studies was reported to be part of the protocol.

On the other hand, Cosentino et al (1983) were able to demonstrate a reduction in sensory nerve conduction velocity in response to a 10-minute treatment duration, compared with five minutes for the other studies (see Table 2.6). Cosentino et al (1983) suggested that the longer duration of exposure to ultrasound is a critical factor for inducing changes to the sensory nerve. Cosentino et al (1983) claimed that the issue, perhaps, is not whether ultrasound has any direct effect on nerve conduction velocities, but rather, at what dosage parameters (frequency, intensity and duration) reductions in sensory nerve conduction velocities sufficient to produce pain relief, occur. It seems that a better understanding of the relevant dosage parameters would be important prior to undertaking clinical trials. Nevertheless, there is an abundance of clinical studies that investigate the effectiveness of ultrasound in producing pain relief which have been undertaken in advance of the issue of dosimetry being resolved.

b. Phase 1 and 2 studies

Randomised clinical trials that have investigated the effects of therapeutic ultrasound on painful conditions are summarized in Table 2.7.

Table 2.7: Summary of Phase 2 Randomised Controlled Trials investigating the effect of ultrasound for pain relief

Authors	Diagnosis	Frequency / Duration	Intensity (W/cm^2)	Outcome measure	Outcome
Creates 1987	Painful perineum (39 Exp vs 37 Pla)	1 & 3 MHz / according to PT	Pulsed, according to PT	VAS pain scale	Exp group improved significantly
Grant et al 1989	Perineal trauma (140 Exp vs 139 Pla)	3 MHz / 6 to 18 minutes	Pulsed (20%). SATP 0.5, SATA 0.1	Pain, oedema	No significant difference between groups
Everett et al 1992	Perineal pain (37 Exp vs 32 Pla)	3 MHz / 5 minutes	Pulsed (50%), SATP 0.5, SATA 0.25	Subjective questionnaire	Exp group had favorable outcome, but this was not significant
Nwuga 1983	Prolapsed Intervertebral disc (27 Exp vs 25 Pla vs 29 Con)	? MHz / 10 minutes	Cont, SATP 1.0 to 2.0	Pain, ROM	Exp group improved significantly
Inaba & Piorkowski 1972	Painful shoulders in hemiplegia (10 Exp vs 10 Pla vs 13 Con)	? MHz / 10 minutes	Cont, SATP 0.5 to 2.0	ROM	No significant difference between groups
Gam et al 1998	Myofascial pain (18 Exp vs 22 Pla vs 18 Con)	0.1 MHz / 3 to 15 minutes	Pulsed (20%), SATP 3.0, SATA 0.6	Pain	Exp and Pla groups significantly better than Con, but no difference between Exp and Pla
Crawford & Snaith 1996	Heel pain (13 Exp vs 13 Pla)	3 MHz / 8 minutes	Pulsed (20%), SATP 0.5, SATA 0.1	VAS pain scale	Exp group had favorable outcome, but this was not significant
Gray et al 1995)	TMJ pain (30 Exp vs 26 Pla)	3 MHz / 2 minutes	Pulsed (66%), SATP 0.25, SATA 0.16	Pain, trismus	Exp group improved significantly
Hasson et al 1990	DOMS (6 Exp vs 6 Pla vs 6 Con)	1 MHz / 20 minutes	Pulsed (20%) SATP 0.8, SATA 0.16	Pain, strength	Exp was significantly better than Pla or Cont
Stay et al 1998	DOMS (12 Exp vs 12 Pla)	1 MHz / 7 minutes	Pulsed (20%), SATP 1.5, SATA 0.3	Pain, swelling, ROM, strength	No significant difference between groups.
Plaskett et al 1999	DOMS (10 Exp vs 10 Pla)	1 MHz / 8 minutes	Pulsed (20%), SATP 1.0, SATA 0.2	Pain, strength	No significant difference between groups
Craig et al 1999	DOMS (12 Exp vs 12 Pla vs 12 Con)	1 MHz / 7 & 15 minutes	Pulsed (20%), SATP 0.8, SATA 0.16	VAS pain scale, McGill pain questionnaire	No significant difference between groups

Exp = Experimental group; Pla = Placebo group; Con = Control group; SATP = Spatial Averaged Temporal Peak; SATA = Spatial Averaged Temporal Averaged; Cont = Continuous, DOMS = Delayed onset muscle soreness; VAS = Visual Analogue Scale; ROM = Range of motion

The first three studies (Creates 1987, Grant et al 1989, Everett et al 1992) investigated the effects of ultrasound on acute pain arising from perineal trauma such as an episiotomy wound following childbirth (see Table 2.7). All three studies employed the pulsed mode; the latter two at 0.5 Watts/cm^2 at 20% (SATA 0.1 Watts/cm^2) and 50% (SATA 0.2 Watts/cm^2) duty factor respectively. The first study (Creates 1987) allowed free selection of the intensity (pulsed mode, duty factor unspecified) by the therapists (Creates 1987). Only the first study by Creates (1987) reported proper calibration of the ultrasound machine during the study, whereas the other two studies reported only that the ultrasound output was checked, but no proper calibration was carried out at any time during the study. Results from these three studies were inconsistent, with the study by Creates (1987) demonstrating a significant improvement in pain in the experimental group. It is possible that the other two studies (Grant et al 1989, Everett et al 1992) did not demonstrate any effect on pain because of the inappropriate selection of treatment dosages, coupled with the lack of calibration of the ultrasound machine during the study.

In a double-blind randomized controlled trial involving 73 subjects with low back pain from prolapsed intervertebral discs, a significant reduction in back pain in the experimental group compared with the placebo and control groups was demonstrated following treatment with therapeutic ultrasound (Nwuga 1983). The dosage used ranged from 1 to 2 Watts/cm^2, continuous mode, for 10 minutes. The unique feature of this study was that the dosage for each subject was calculated based on a formula proposed by Reid and Cummings (1973). This calculation provided a common and consistent guideline for all the treatment sessions and its influence on the

overall positive outcome cannot be underestimated.

In another double-blind randomized controlled trial investigating the effects of ultrasound on chronic painful shoulders in patients with hemiplegia (Inaba and Piorkowski 1972), no significant difference was found between the experimental, placebo and control groups. However, this study had several weaknesses. Firstly, while the therapists were free to choose the treatment intensity for each treatment session (ranging from 0.5 to 2 Watts/cm^2, continuous mode), it was determined solely by patient tolerance. No proper guidelines were provided and this could possibly lead to inconsistencies in the selected treatment intensity between sessions as well as between patients. Secondly, the ultrasound machine was not calibrated during the entire study, casting further doubt on the issue of dosimetry. In addition, the outcome measure chosen may have been inappropriate. The investigators made an assumption that a reduction in pain was reflected by a change in the range of motion in the shoulder. As such, the subjects' shoulder range of motion, and not pain, was the main dependent variable measured. However, all three groups were given range of motion exercises, and as a result, all three groups improved their shoulder range of motion. It can be argued that the purpose of this study (to investigate the effect of ultrasound on shoulder pain) was never achieved since pain was never measured. The indirect measurement of improvement in pain via changes in range of motion could not be evaluated as range of motion exercises were prescribed for all three groups. The conclusion of this study, that ultrasound was ineffective in reducing shoulder pain in hemiplegic patients, could not be supported based on the reported results. Yet, this study is often included in systematic reviews and cited as evidence

to indicate the lack of effectiveness of ultrasound (Holmes and Rudland 1991, Gam and Johannsen 1995, van der Windt et al 1999).

Gam et al (1998) investigated the effects of ultrasound on myofascial trigger points. The experimental group received ultrasound, massage and exercises, while the placebo group received sham ultrasound, massage and exercises. The control group received no treatment whatsoever. The ultrasound machine was supplied and calibrated by its manufacturer. Dosage was standardized at 3 Watts/cm^2, pulsed at 20% (or SATA 0.6 Watts/cm^2) for three minutes for each trigger point (up to a maximum of 5 trigger points for treatment). The results showed that both the experimental and placebo groups were significantly better than the control group, but there was no difference between the experimental and placebo groups. The authors concluded that ultrasound was unable to reduce pain arising from myofascial trigger points. Massage and exercise, on the other hand, were considered more effective than ultrasound in the treatment of myofascial trigger points.

The next two studies are not strictly RCTs as they lack a control group in their design. The effect of ultrasound on heel pain (Crawford and Snaith 1996) and temporomandibular pain (Gray et al 1995) were investigated. In addition, Gray et al (1995) also evaluated the effects of three other commonly used electrophysical agents: short-wave diathermy, megapulse, and laser. Crawford and Snaith (1996) reported that there was improvement in the experimental group, but this was not significant when compared with the placebo group. The machine was calibrated and the dosage used was fairly conservative (SATA 0.1 Watt/cm^2). It is possible that the authors would have been able to obtain a better result with a

different dosage. On the other hand, Gray et al (1995) were able to demonstrate a significant difference between the experimental and placebo groups with an ultrasound intensity of 0.16 Watts/cm^2 (SATA), although there were no differences among the four types of treatment methods (ultrasound, short-wave diathermy, megapulse, and laser) evaluated.

The last four studies in Table 2.7 pertain to studies investigating the effects of ultrasound on delayed onset muscle soreness (DOMS). Delayed onset muscle soreness, though technically not an injury is caused by the build-up of lactic acid in the muscles following strong bouts of exercise (Hasson et al 1990). The reabsorption of lactic acid through the circulation takes a few days, and the reduction in pain is concurrent with this reabsorption. The rationale for using ultrasound in the management of DOMS is to increase the rate of reabsorption of lactic acid via increasing circulation to the muscles (Hasson et al 1990). While the evidence regarding changes to circulation caused by exposure to ultrasound is ambiguous, clinical trials hoping to achieve this effect have been conducted despite experimental results showing that it is still controversial. Three of the four studies show that ultrasound was not effective (Stay et al 1998, Plaskett et al 1999, Craig et al 1999). The one study that had a positive outcome, however, only had 6 subjects in each group and this could have affected the results (Hasson et al 1990).

(3) Summary

In a critical review of ultrasound in the treatment of musculoskeletal conditions, Falconer et al (1990) concluded that the evidence for pain relief was inconclusive. These authors claimed that it was not possible to identify whether the ultrasound was administered

appropriately since inadequate information had been provided in the published reports. In contrast, Gam and Johannsen (1995) in their meta-analysis were more decisive. They concluded that pain relief could not be achieved by ultrasound treatment, and that a dose-response relationship could not be clarified due to inadequate information on treatment area and size of the transducer. From the RCTs summarized in Table 2.7, it is clear that both positive and negative results have been obtained, a conclusion that is consistent with the available pre-clinical studies. The positive outcome studies did have some common characteristics; their treatment duration was more than 10 minutes and they employed rather high intensities, with a few exceptions (Hasson et al 1990, Gray et al 1995). There is some suggestion that the effect on sensory nerves and circulation is dose-dependent and this is also the trend seen in pre-clinical studies. Exactly what the dose-dependent relationship is, however, remains to be resolved, and the evaluation of clinical studies prior to this must be considered premature.

c. Facilitation of tissue healing

Ultrasound has been used to enhance tissue healing in both soft tissues such as skin, tendon, and muscle; as well as hard tissues such as bone (Warden et al 1999). However, the use of ultrasound in bone healing involves specially modified diagnostic ultrasound equipment with very low output (in milliwatts); something that is quite different from therapeutic ultrasound units (Warden et al 1999). Hence, the discussion of hard tissue healing is not within the scope of this review. This section will describe soft tissue healing in skin, tendon, ligament, muscle and nerve.

It has been suggested that soft tissue repair may be accelerated by both the

thermal and non-thermal effects of ultrasound (Lehmann et al 1978, Dyson 1987). However, when healing is already occurring at an optimal level, ultrasound may not affect an increase (McDiarmid et al 1985, Snow and Johnson 1988).

The mechanism for tissue healing follows on from the acute inflammatory process; that is, the proliferation phase and the remodeling maturation phase. The proliferation phase begins around the 3rd to 5th day after injury, and lasts for approximately three weeks (Maxwell 1992). Collagen synthesis and turnover peaks during this phase, with the type I collagen predominating over the type III. The remodeling maturation phase begins around the third week, with some overlap from the previous proliferation phase (Maxwell 1992). Remodeling can take up to several months or years, depending on the extent of the initial injury, until the collagen regains the same pre-injury pattern and mechanical characteristics of the tissue (Maxwell 1992). This usually occurs in response to the mechanical stresses imposed on the tissues.

Exposure to low intensity therapeutic ultrasound has been shown to induce the release of mitogenic factors from platelets, macrophages and mast cells, as well as the stimulation of fibroblastic activity, which enhances calcium influx, cell proliferation, and the synthesis of collagen (Houghton 1999). Studies have shown that ultrasound can facilitate the rate of healing of tissues as well as the quality of the scar tissue formed (Houghton 1999).

(1) Initial studies

Byl et al (1993) used an *in vivo* pig model to investigate the effects of two different intensities of ultrasound on wound healing; low dose (pulsed, SATA 0.1 Watts/cm^2) and high dose (continuous, SATP 1.5 W/cm^2). Their results (see Table 2.8) demonstrate that during the first week (acute inflammation phase) low and high dose ultrasound were able to effectively

Table 2.8: Summary of initial studies investigating the effect of ultrasound on tissue healing

Author	Type of Study	Frequency / Duration	Intensity (W/cm^2)	Effect on Tissue Healing
Byl et al 1993	Skin wound (Pig *in vivo*)	1 MHz / 5 minutes	Cont, SATP 1.5 (HUS)	LUS or HUS for 1 week enhances wound breaking strength in acute wounds; For facilitating wound strength and collagen deposition, LUS should be used for 2 weeks or more
			Pulsed (20%), SATP 0.5 (LUS) SATA 0.1	
Rantanen et al 1999	Muscle (rat *in vivo*)	3 MHz / 6 minutes; stationary head	Pulsed (20%), SATP 1.5, SATA 0.3	No overall significant effect on muscle regeneration
Enwemeka 1989	Tendons (rabbits *in vivo*)	1 MHz / 5 minutes	Cont, SATP 1.0	Increased tensile strength and energy absorption capacity of tendons was achieved
Huang et al 1999	Cartilage (rat *in vivo*)	1 MHz / 7 minutes	Pulsed (25%), SATP 2.5, SATA 0.625	Repair of cartilage was significantly enhanced in Exp group

SATP = Spatial Averaged Temporal Peak; SATA = Spatial Averaged Temporal Averaged; Cont = Continuous; HUS = High intensity ultrasound; LUS = Low intensity ultrasound.

enhance wound-breaking strength equally. However, after 2 weeks (proliferation phase), low dose ultrasound was more effective at enhancing wound strength as well as collagen deposition. In these experiments, a dose-response relationship was clearly demonstrated, as well as a relationship between the dose and the stages of tissue healing. The low dose selected for this study (SATA 0.1Watt/cm^2) might appear to be extremely conservative when compared with dosages commonly applied to treat tissues such as muscles and tendons. However, as the target tissue in this study was superficial (skin wound) almost all of the energy would be easily delivered to the target tissue.

On the other hand, target tissues such as muscles, which are situated more deeply, would require a significantly higher dosage to achieve similar effects. Rantanen et al (1999) investigated the effects of ultrasound

on muscle regeneration following injury in an *in vivo* rat model (Table 2.8). Their results demonstrated that there was no significant effect on muscle regeneration compared with the control group. A dosage of 3MHz, pulsed SATA 0.3 Watts/cm^2, for 6 minutes was used. A stationary sound head technique was used and it is possible that the deleterious effects of the standing waves produced in such a technique (Dyson 1987) may have confounded their results.

Enwemeka (1989) investigated the effects of therapeutic ultrasound on tendon healing in an *in vivo* rabbit model (Table 2.8). The dosage used in the study was 1 MHz, continuous, at 1.0 Watt/cm^2, for five minutes daily. As an underwater in-direct application technique was used, the energy reaching the target tissue would be attenuated and vary according to the distance between the transducer and the skin (Robertson and Ward 1996). Nevertheless, the ultrasound insonation was able to increase both the tensile strength and the energy absorption capacity of the experimental group. Similarly, positive effects of ultrasound on articular cartilage repair have been demonstrated using an *in vivo* rat model (Huang et al 1999). The dosage selected for their study was 1 MHz, pulsed, SATA 0.625 Watts/cm^2, for 7 minutes (Table 2.8).

(2) Phase 1 and 2 studies

Clinical trials investigating the effects of ultrasound on tissue healing are summarized in Table 2.9. Only studies investigating tissue healing in nerves and skin were identified. No studies were found related to other soft tissues such as tendons, muscle and cartilage.

Table 2.9: Summary of Phase 2 Randomised Controlled Trials investigating the effect of ultrasound on tissue healing

Authors	Diagnosis	Frequency / Duration	Intensity (W/cm^2)	Outcome measure	Outcome
Ebenbichler et al 1998	Carpal tunnel syndrome (34 Exp vs 34 Pla)	1 MHz / 15 minutes	Pulsed (20%), SATP 1.0, SATA 0.2	Pain, electro-neurography	Exp group improved significantly
Oztas et al 1998	Carpal tunnel syndrome (10 Exp vs 10 Pla)	3 MHz / 5 minutes	Cont, SATP 0.8 & 1.5	Pain, electro-neurography	Exp group had favorable outcome, but this was not significant
Dyson & Suckling 1978	Venous ulcers (7 Exp vs 7 Pla)	3 MHz / 5 to 10 minutes	Pulsed (20%), SATP 1.0, SATA 0.2	Healing rate	Exp group improved significantly compared to placebo
Roche & West 1984	Venous ulcers (13 Exp vs 13 Pla)	3 MHz / 5 to 10 minutes	Pulsed (20%), SATP 1.0, SATA 0.2	Healing rate	Exp group improved significantly compared to placebo
Callam et al 1987	Chronic leg ulcers (52 Exp vs 56 Con)	1 MHz / 1 minute	Pulsed (10%), SATP 0.5, SATA 0.05	Healing rate	Exp group improved significantly compared to controls
Weichenthal et al 1997	Chronic venous ulcers (19 Exp vs 18 Con)	30 kHz / 10 minutes	Cont, SATP 0.01	Healing rate	Exp group improved significantly compared to controls
McDiarmid et al 1985	Pressure sores (21 Exp vs 19 Pla)	3 MHz / 5 to 10 minutes	Pulsed (20%), SATP 0.8, SATA 0.16	Healing rate	No difference for clean wounds, faster rate for infected wounds
Lundeberg et al 1990	Venous ulcers (22 Exp vs 22 Pla)	1 MHz / 10 minutes	Pulsed (10%), SATP 0.5, SATA 0.05	Healing rate	No significant difference between groups
Eriksson et al 1991	Chronic leg ulcers (19 Exp vs 19 Pla)	1 MHz / 10 minutes	Cont, SATP 1.0,	Healing rate	No significant difference between groups
ter Riet et al 1996	Pressure ulcers (45 Exp vs 43 Pla)	3.28 MHz / 3 to 10 minutes	Pulsed (20%), SATP 0.5, SATA 0.01	Healing rate	No significant difference between groups

Exp = Experimental group; Pla = Placebo group; Con = Control group; SATP = Spatial Averaged Temporal Peak; SATA = Spatial Averaged Temporal Averaged; Cont = Continuous

Two studies investigated the effects of ultrasound on healing of nerve tissue in carpal tunnel syndrome. Nerve tissues are rich in protein and as such, are very good absorbers of ultrasonic energy. In addition, the carpal tunnel is very superficially sited and should be easily accessible to the ultrasonic energy. The dosages for both groups appear to be comparable and appropriate: SATA 0.2 Watts/cm^2 for 15 minutes (Ebenbichler et al 1998) and SATP 0.8 and 1.5 Watts/cm^2 for 5 minutes (Oztas et al 1998). The former chose a low intensity with long duration of exposure to adjust for the 1MHz frequency chosen; while the latter chose a shorter duration of

exposure to adjust for the higher intensities chosen. Ebenbichler et al (1998) reported that the experimental group improved significantly, compared with the non-significant improvement in the experimental group reported by Oztas et al (1998). The results from Oztas et al's study (1998) could have been influenced by the low number of subjects in each group (10) compared with 34 in each group reported by Ebenbichler et al (1998).

The next eight studies all dealt with tissue healing in skin ulcers. Of these, 5 reported increased healing rates compared with a control group (Dyson and Suckling 1978, Roche and West 1984, Weichenthal et al 1997, Callam et al 1987, McDiarmid et al 1985), while the other three reported no significant differences between the experimental and control or placebo groups (Lundeberg et al 1990, Eriksson et al 1991, ter Riet et al 1996).

Houghton (1999) claimed that the conflicting results were due to lack of statistical power in the negative reports, as well as the variability in the patient populations. Houghton (1999) recommended that given the positive results of clinical trials performed since 1995, as well as the results of a meta-analysis by Johannsen et al (1998), the use of ultrasound in chronic wound healing is justified.

(3) Summary

The evidence for soft-tissue healing in ulcers seems to be quite convincing (Houghton 1999). However, based on the initial studies reported (Table 2.8), the potential for tissue healing in muscles, tendons, and cartilage, has not been adequately investigated and more clinical trials in these areas are needed.

d. Changes in tissue extensibility

Connective tissue can be classified into 4 groups (Lehmann et al 1970): loose (e.g. subcutaneous tissue), dense (e.g. fascia and muscle), organized (e.g. ligaments and tendons), and specialized (e.g. cartilage and bone). Generally, connective tissues are viscoelastic structures capable of both plastic and elastic changes under certain conditions (Lehmann et al 1970). The viscous properties of connective tissues enable it to undergo a permanent change in structure (elongation or shortening), whereas the elastic properties enable it to regain its original length (Lehmann et al 1970, Warren et al 1971, 1976). The amount of elastic and viscous deformation varies, depending on the amount and duration of the force applied, and the tissue temperature (Lehmann et al 1970, Warren et al 1971, Warren et al 1976). In clinical conditions where shortening of dense (e.g. fascia and muscle) and organized (e.g. ligaments and tendons) connective tissue occurs, it is theoretically possible to stretch the elastic components of the tissue to regain its normal length. Heating of the tissues, prior to stretching, will facilitate their recovery and appears to be the rationale for the use of therapeutic ultrasound in these conditions.

(1) Initial studies

Lehmann et al (1970) investigated the effects of stretching a collagenous tissue, such as a tendon from a rat's tail, under various conditions; heating alone, stretch alone, heating prior to stretch, heating during the stretch, and short term sustained and cyclic stretching (Table 2.10). Their study showed that heating or stretching alone did not produce elongation of the rat tail tendon. However, combined heating (at 45°C) and stretching produced the best and significant residual length increase in the tendon. Heating was achieved by immersion of the tendon tail in a hot water bath.

Table 2.10: Summary of initial studies investigating the effect of ultrasound on tissue extensibility

Author	Type of Study	Frequency / Duration	Intensity (W/cm^2)	Effect on tissue extensibility
Lehmann et al 1970	Tendons (rat *in vitro*)		45℃ and 25℃	Most effective way of treating contractures was to apply heat while applying sustained stretch and to maintain stretch well after loading period to achieve elongation
Warren et al 1976	Tendons (rat *in vitro*)		37℃	Low force, long duration procedure was effective at producing residual elongation. Heating prior to loading was found to cause less damage.
Wessling et al 1987	Muscle (human *in vivo*)	? MHz / 7 minutes	Cont, SATP 1.5	Static stretch + US combined increased ankle dorsiflexion more than static stretch alone
Draper et al 1998b	Muscle (human *in vivo*)	3 MHz / 7 minutes	Cont, SATP 1.5	Immediate effects: preheat + stretch increases ROM significantly compared to stretch alone; Residual effects: no significant difference between 2 groups
Reed & Ashikaga 1997	Ligament (human *in vivo*)	1 MHz / 8 minutes	Cont, SATP 1.5	Increased extensibility in some ligaments (heat only, no stretch)
Reed et al 2000	Ligament (human *in vivo*)	3 MHz / 2.5 minutes	Cont, SATP 1.25	Heat and stretch was not significantly different from stretching alone

SATP = Spatial Averaged Temporal Peak; Cont = Continuous

In a similar experiment, Warren et al (1976) evaluated various methods of applying force to collagenous tissues (rat tail tendon) at various temperatures to achieve permanent elongation (Table 2.10). The results demonstrated that a low load, long duration procedure was most effective at producing residual elongation of the tendon. In addition, heating and maintaining the tissue at elevated temperatures prior to applying the load was found to cause significantly less damage.

Wessling et al (1987) demonstrated in his group of healthy subjects that static stretch combined with therapeutic ultrasound increased gastrocnemius muscle extensibility more than static stretch alone (Table

2.10). The ultrasound dosage used was quite high (continuous, 1.5W/cm2) applied for 7 minutes over the muscle belly. The frequency of the ultrasonic machine used was not reported.

Draper et al (1998b) achieved similar results with exactly the same dosage as that reported by Wessling et al (1987) (Table 2.10). The frequency of the ultrasonic machine used by Draper et al (1998b) was 3 MHz. The results showed that the heat and stretch program was able to increase the muscle extensibility significantly. The long-term effects, however, were not significantly different.

Reid and Ashikaga (1997) demonstrated increased extensibility in ligaments, rather than muscles, by using the same dosage (continuous, 1.5Watts/cm^2) as Wessling et al (1987) with a 1 MHz ultrasound machine for eight minutes (Table 2.10). However, Reid et al (2000) failed to demonstrate the same results when they used a 3 MHz ultrasound machine with a very short exposure time (2.5 minutes). The decrease in exposure time (from 8 minutes to 2.5 minutes) could be the major reason why the second study (Reid et al 2000) did not replicate the positive results of the first study (Reid and Ashikaga 1997).

It is possible that all the pre-clinical studies on normal subjects were able to show positive results in increasing tissue extensibility in tendons, muscles and ligaments due to appropriate dosages being selected. In order for the viscous component of the tissue to be affected, it has to be heated sufficiently by the ultrasonic energy.

Table 2.11: Summary of Phase 2 Randomised Controlled Trials investigating the effect of ultrasound on tissue extensibility

Authors	Diagnosis	Frequency / Duration	Intensity (W/cm^2)	Outcome measure	Outcome
Falconer et al 1992	OA Knee (34 Exp vs 35 Con)	1 MHz / 12 minutes	Cont, SATP variable	Pain, ROM	No difference between groups
Ward et al 1994	Scar contracture (8 Exp vs 6 Pla)	1 MHz / 10 minutes	Cont, SATP 1.0	ROM	No difference between groups

Exp = Experimental group; Pla = Placebo group; Con = Control group; SATP = Spatial Averaged Temporal Peak; Cont = Continuous; ROM = Range of motion

(2) Phase 1 and 2 studies

When compared with initial studies, phase 1 and 2 studies investigating the effects of ultrasound on tissue extensibility are limited. These studies are summarized in Table 2.11.

Falconer et al (1992) investigated the effects of ultrasound on mobility of the knee in patients with osteoarthritis. The dosage parameters were 1 MHz frequency, for 12 minutes exposure to ultrasound in the continuous mode. The intensity was variably adjusted, depending on patient's tolerance. Their results demonstrated no difference between the experimental and control groups.

Ward et al (1994) investigated the effects of ultrasound on scar tissue extensibility. Their subjects were patients with scar tissue contractures secondary to burns. Therapeutic ultrasound at 1MHz, continuous, at 1.0 Watts/cm^2 was applied to the scar tissue for 10 minutes, followed by another 10 minutes of passive stretching. Their results demonstrated no significant benefit in either range of motion or pain. It can be argued that in such acute cases where pain is still a major factor in the burn patients, the results of the study could have been affected by the patients' pain perception. It may have been more appropriate, perhaps, to

perform the study on a more chronic group of patients where pain was no longer a confounding variable.

(3) Summary

The possibilities for increasing tissue extensibility with therapeutic ultrasound seem to need further investigation. The initial studies do indicate that there is a potential for the clinical application of ultrasound in this area. However, dosimetry and procedural issues have only been partially answered in initial studies and would need to be investigated in a patient population.

2.7 ADVERSE REACTIONS / TISSUE DAMAGE

While there have been few documented cases of adverse reactions to treatment with therapeutic ultrasound (Kitchen 2000), the application of therapeutic ultrasound is not without some risks. Textbooks generally compile lists of conditions and situations where the application of therapeutic ultrasound is contraindicated and this includes application over the eyes, in the presence of defective thermal sensation, over tumors, the pregnant uterus, among others (Low and Reed 2000). Not all the contraindications, however, are based on experimental evidence, and many have never been evaluated.

One exception to this lack of evidence is the effect of therapeutic ultrasound on tumors. Sicard-Rosenbaum et al (1995, 1998) were able to demonstrate in a rat model, that therapeutic ultrasound can cause tumor cells to grow, and that this growth was both dose-dependent and similar for continuous or pulsed modes. The authors cautioned that therapeutic ultrasound should not be applied over tumors or suspected tumors. In addition, Houghton and Radman (2000) were able to demonstrate that therapeutic ultrasound could affect the development of fetal limbs in a rat organ

culture system. While the results from these *in vitro* studies on small animals do not necessarily translate directly to the human *in vivo* situation, they do serve to indicate that caution in the use of therapeutic ultrasound should be applied as ultrasound has the potential to cause harm if not applied appropriately.

In the proposed research model (Figure 2.2, p44), adverse effects of therapeutic modalities are investigated in Phase 1 studies. Gill et al (1992) proposed that in order to evaluate potential adverse effects of interventions, a single subject design is useful as an alternative to Phase 1 type of studies. To date, there have been few, if any, documented case reports involving adverse effects from any therapeutic interventions, including therapeutic ultrasound (only 1%, see Figure 2.3, p46). The scarcity of these case reports may not be a reflection of the safety of the interventions, but rather an indication that physiotherapists are reluctant to evaluate their safety record, since it may have medico-legal implications. Only two case reports on adverse reactions to therapeutic ultrasound were found, and medical practitioners, rather than physiotherapists reported both of these (Levenson et al 1983, Gnatz 1989). The first case report involved a woman who self-administered ultrasound over a 2 year period, and subsequently developed chronic pain and bleeding (Levenson et al 1983). The second report documented increased pain from the application of therapeutic ultrasound to the back in two patients with lumbar disk herniation. Interestingly, the ultrasound equipment itself was never suspected to be the cause of the adverse reaction and was never reported as having been sent for checking. As has been pointed out already, the output from therapeutic ultrasound equipment has been shown to be unreliable and is a source of potential danger if not checked regularly.

MacDonald and Shipster (1981) cautioned that there is insufficient information regarding the temperatures reached in different tissues, and the extent to

which temperatures at dangerously high levels are capable of damaging the tissues. This issue must be addressed in order to establish the upper safety limit of intensities for both clinicians and researchers when attempting to select an appropriate treatment dosage.

2.8 SUMMARY

- The usage of therapeutic ultrasound is one of the highest of all the available electrophysical agents, although there seems to be a downward trend in usage following some negative reviews in the literature (Goh et al 1999).
- The determination of treatment dosages in therapeutic ultrasound requires further clarification. In the absence of concrete guidelines based on experimental data, the selection of treatment dosages are associated with factors such as the clinician's gender, clinical specialty, country of training, and educational background (Goh et al 1999).
- The two main considerations when determining if a treatment dose is appropriate is whether sufficient ultrasonic energy has reached the target tissues, and whether the same energy can be delivered to the same target tissues consistently.
- Increase in temperature at the target tissue is a useful indication that the ultrasonic energy can be successfully delivered to the target tissue.
- Factors to consider when making a selection of treatment dosage for ultrasound include frequency (ter Haar 1987), intensity (Young and Dyson 1990) and duration of exposure (Oakley 1987).
- When attempting to deliver ultrasonic energy to the target tissues, the depth of the target tissue is a primary consideration. Due to attenuation of the energy, loss of energy occurs as the tissue depth increases (ter Haar 1987).

The half value distance for 3 MHz is 1.5 cm and for 1 MHz is 5 cm (Wells 1977). Therefore, in order to fully appreciate the heating pattern that occurs in the tissues and to account for all possible target tissues in most clinical applications, measurements of temperature increases up to 5 cm below the skin surface can be considered appropriate.

- As long as the thickness of fat below the skin is less than 3cm, the heating pattern in the deeper structures will be unaffected (Draper and Sunderland 1993).
- Commercially available ultrasonic gel is the most suitable coupling agent for use (Williams 1987).
- Factors that will ensure a consistent and reproducible delivery of the ultrasonic energy to the target tissues include:
  - Calibration of intensity output to within $\pm$ 10% error is necessary.
  - Calibration of intensity output at least once every four weeks.
  - Use of direct in-contact technique with gel rather than other techniques.
  - Maintaining the transducer of the ultrasound perpendicular to the target tissues at all times.
  - Ensuring the coupling agent is at room temperature to provide maximum transmissivity.
  - Maintaining a constant pressure of the transducer on the tissues as it can affect the heating pattern produced in the target tissues. It is recommended that firm pressure is adequate and the weight of the transducer head is probably sufficient.
  - Maintaining the size of the treatment area constant, as this will have an effect on the heating pattern produced in the target tissues. For

3 MHz frequency, a treatment area corresponding to twice the size of the transducer is recommended (Chan et al 1998). For 1 MHz frequency, it is assumed to be the same although this may require further investigation.

- Moving the transducer throughout the treatment time in order to avoid the formation of standing waves in the tissues. Exactly how fast the transducer should be moved is unknown, and may require further investigation.
- Examining and reporting the reliability and reproducibility of any investigative procedure used.

In order to investigate the relationship between frequency, intensity and duration of exposure on temperature increase in the target tissues, an *in vitro* pig model with access to the full cross-section of the heating pattern can be considered appropriate. To measure both point as well as area temperatures from the exposed cross-section of tissues, infrared thermography has been identified as a suitable instrument. A review of the biophysical and clinical studies demonstrates that improper selection of treatment dosages could be one factor contributing to inconsistent outcomes. This emphasizes the need to develop a common model for determining treatment dosages that can be used to guide both clinicians and researchers in the use of therapeutic ultrasound. In addition, there is a need to determine the upper safety limit of intensities used in therapeutic ultrasound.

CHAPTER THREE

METHODOLOGY

3.1 INTRODUCTION

This study comprised a series of experiments with the ultimate purpose of examining the relationship between ultrasound dose (as determined by frequency, intensity and duration of exposure) and depth of tissue heating. While each of the experiments differed slightly in tissue specimens, instrumentation, procedure and data analysis, a numerous aspects of the procedure were common to all. Common features in methodology and treatment parameters from each of the experiments are summarized in Figure 3.1 and Table 3.1 respectively, and are described in this chapter. Specific hypotheses and procedural details are described in the subsequent chapters with the results of the individual investigations.

3.2 TISSUE SPECIMENS

The experiments required an animal model with a skin structure and skin-fat-muscle-bone proportions similar to humans. Tissue specimens from small animals such as rats and rabbits were, therefore, not suitable as experimental specimens. Adult domestic pigs were considered as being able to meet these requirements and have been used in research involving therapeutic ultrasound in the past (see Chapter 2, Section 2.5, p36). The adult domestic pig closely resembles the human in terms of skin, fat, muscle and bone composition (Lavker et al 1991) and is considered an appropriate alternative model for these types of investigations.

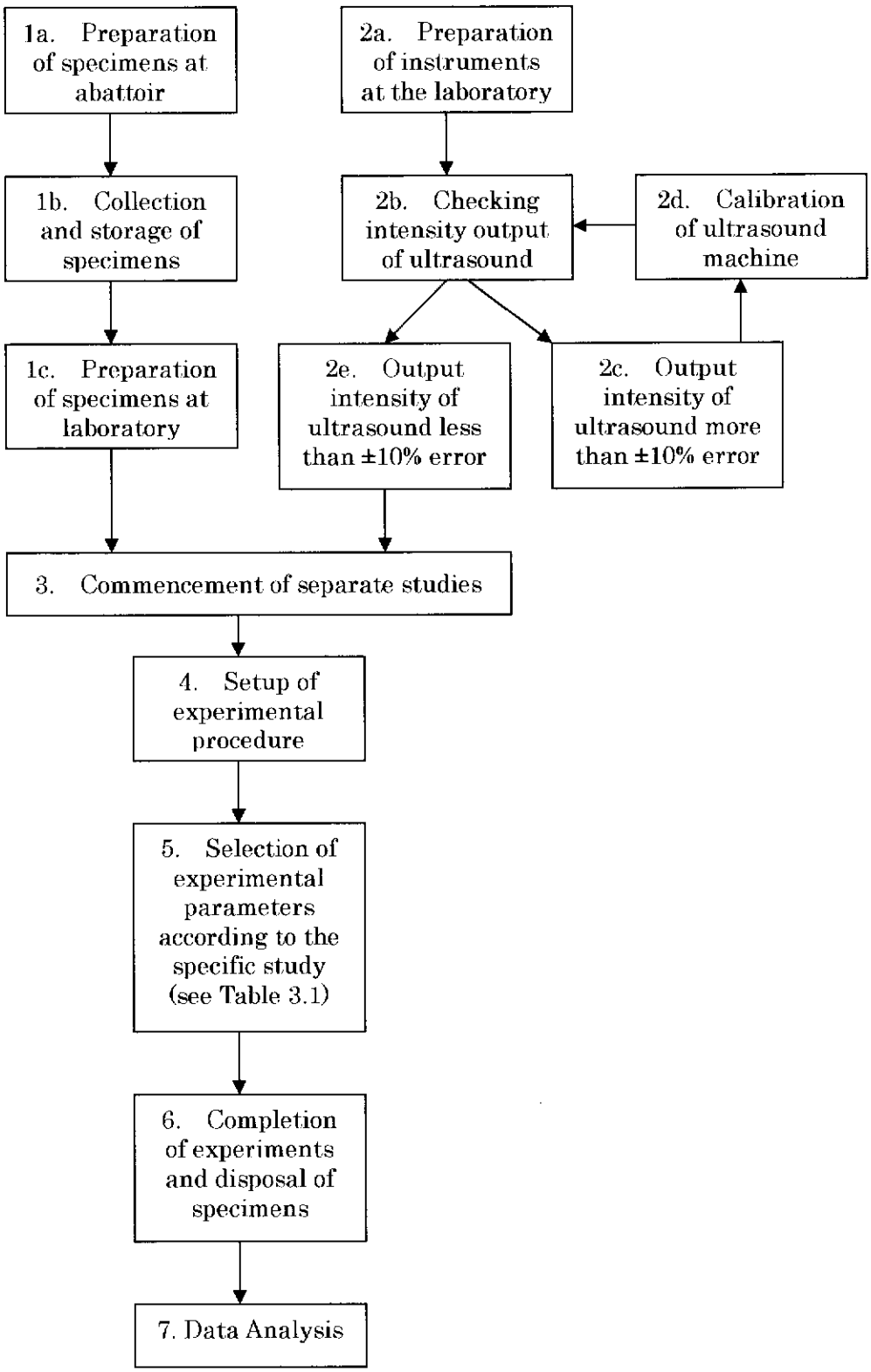

Figure 3.1: Flow chart of methodology common to all studies

Table 3.1: Selection of experimental parameters, type of data analysis and number of specimens for specific studies

Treatment Parameters	Pre-study	Procedural Study 1	Procedural Study 2	Procedural Study 3	Main Study
	Reliability	Movement speed of transducer	Size of treatment area	Thermal and mechanical cellular damage	Frequency, Intensity and Duration of exposure
Frequency (MHz)	1	1 & 3	1	1 & 3	1 & 3
Intensity (watts/cm^2)	1.0	1.0	1.0	0.5, 1.0, 1.5 (1 & 3 MHz), 2.0 (1 MHz), 0.0 (control)	0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5
Duration (mins)	10 on, 10 off (pre and post session)	10 on, 10 off	10 on, 10 off	10 on	10 on, 10 off
Speed (beats/min)	120	60, 120, 180	120	120	120
Size of treatment area (X ERA)	2 X ERA	2 X ERA	2, 3, 4 X ERA	2 X ERA	2 X ERA
Data analysis	ICCs (single measure, 2-way mixed effect model)	Repeated Measures ANOVA (General Linear Model, Repeated contrasts)	Repeated Measures ANOVA (General Linear Model, Repeated contrasts)	Qualitative analysis	Repeated Measures ANOVA (General Linear Model, Repeated contrasts)
Total no. of specimens	13	20	15	8	20

ERA = Effective Radiating Area; ICCs = Intraclass correlation coefficients; ANOVA = Analysis of variance;

Table 3.2: Performance characteristics of Omnisound 3000™

Frequency	ERA	BNR	Maximum Power Output	Maximum Output Intensity
1 MHz	5.2 cm^2	1.4:1	12 Watts	2.0 Watts/cm^2
3 MHz	5.2 cm^2	3.5:1	9 Watts	1.5 Watts/cm^2

ERA = Effective Radiating Area; BNR = Beam Non-uniformity Ratio

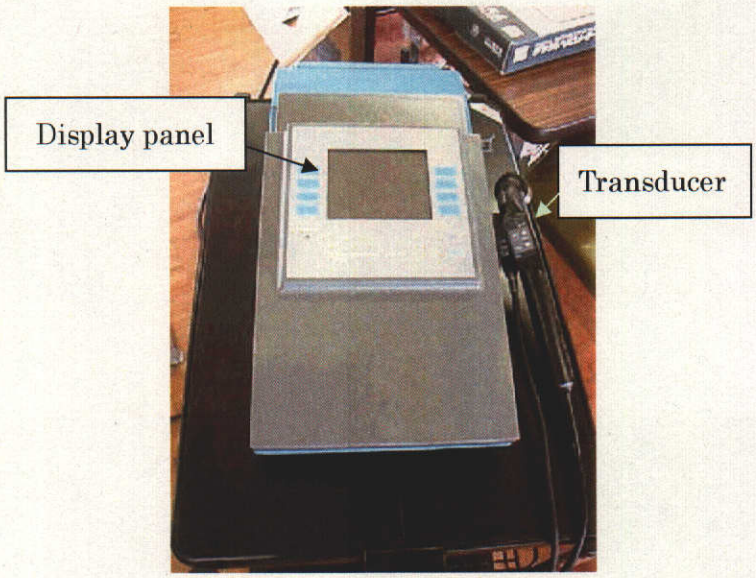

Figure 3.2: The Omnisound 3000™ Therapeutic Ultrasound Unit

3.3 INSTRUMENTATION

a. Therapeutic Ultrasound (Figure 3.2). The therapeutic ultrasound machine used throughout the study was the Omnisound 3000™ (Physio Technology Inc., Topeka, Kansas, USA). This machine has a lead zirconate titanate (PZT) crystal and has the capability to provide ultrasound output at two frequencies: 1 and 3 MHz. Software provided within the machine was used during the calibration procedure to adjust the display output with that of the power meter (see Section 3.4, paragraph f, p97). Output intensity could be adjusted in 0.1 Watt/cm^2 increments. The digital display of the power and output intensity was in Watts or Watts/cm^2 respectively. The in-built digital timer display was in minutes and seconds, and could be adjusted by 30-second intervals. The performance parameters of the Omnisound 3000™ are given in Table 3.2 and the machine is displayed in Figure 3.2.

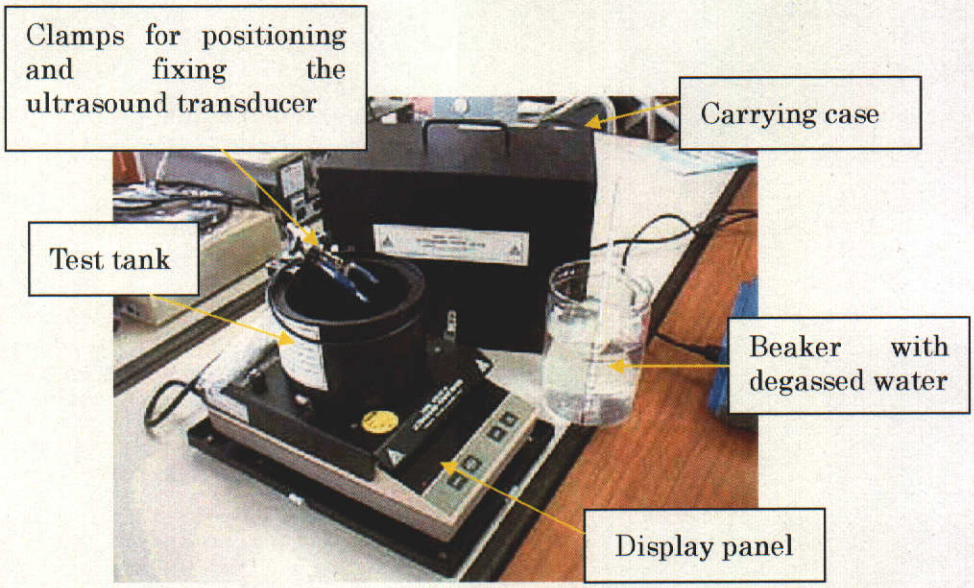

Figure 3.3: Power meter, Model UPM-DT by Ohmnic Instruments

b. Power Meter (Figure 3.3). Output from the Omnisound 3000™ was calibrated prior to each experiment using a power meter (Fig 3.3) from Ohmnic Instruments, Model UPM-DT-10 (Ohmnic Instruments Co., St. Michaels, Maryland 21663, USA) that was factory calibrated and supplied with a certificate of calibration. The display output from the power meter was in Watts.

c. Infrared Video Thermography Unit (Figure 3.4). The Avio thermal video system (TVS) 2000™ (Nippon Avionics Co. Ltd., Japan) was used for all the experiments. The Avio TVS 2000 is a video thermal imaging system with radiometric temperature measurement capability. The image is acquired at 30 Hz and presented in up to 256 colours on the built-in six-inch colour display monitor. The TVS 2000 consists of two main components: the imager (or infrared camera head), and the main processor unit. The imager receives the infrared energy emitted from the surface of an object and converts this into an electrical signal. The processor converts the

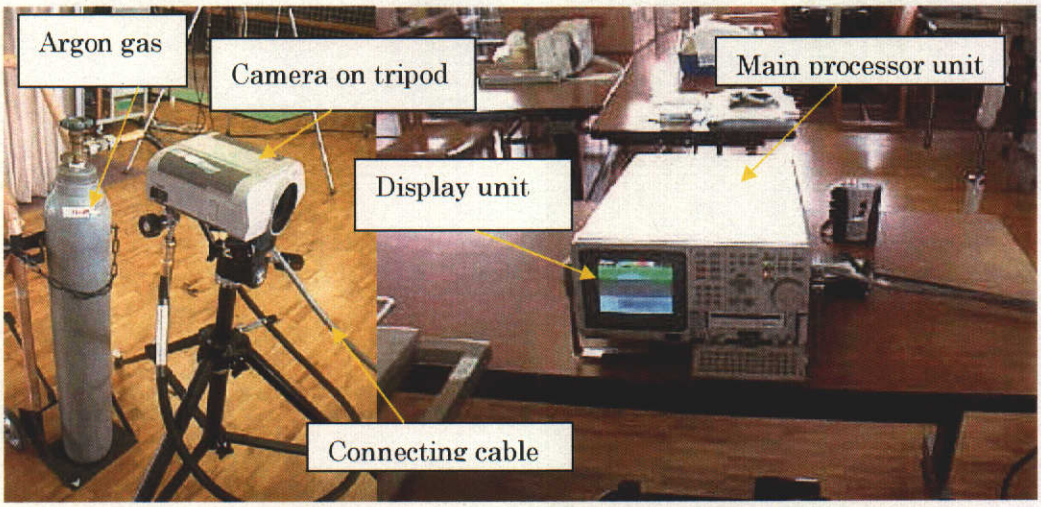

Figure 3.4: Avio Infrared Thermal Video System (TVS) 2000

electrical signal from the imager into a digital signal and stores it in the frame memory. When a complete frame of infrared video information has been acquired, it is displayed. The TVS 2000 offers several built-in image processing and analysis features such as image averaging, selectable number of displayed levels, 2 times zoom display, display freeze, multi-point temperature indication, as well as time, data and user message. The TVS 2000 can also automatically track temperature and record or replay thermal images using data stored on the built-in 3.5 inch disk drive.

The infrared camera was mounted on a standard camera tripod. The main-processor unit was attached to the camera via a parallel cable. The infrared camera was attached to a cylinder of pressurized Argon gas (30°C) that was used as a reference temperature for the camera unit. Figure 3.4 shows the setup of the system. The display unit allowed up to a maximum of five point-markers to be pre-set. The temperature display was in degrees Celsius, up to 0.01°C. Emissivity of the camera was set at 0.98 to correspond to the emissivity for human tissues. The sensitivity and temperature ranges were individually set for each experiment.

Figure 3.5: Minolta Spot Thermometer HT-11

d. Infrared Spot Thermometer (Figure 3.5). The Minolta spot thermometer HT-11 (Minolta Co. Ltd., Japan) was used to measure the skin surface temperature throughout the entire study. The Minolta spot thermometer is a portable non-contact infrared thermo-sensor. It displays the temperature in degree Celsius, in increments of 0.1°C. Emissivity was set at 0.98 to correspond to the emissivity for human tissues.

e. Correlation of Infrared Video Thermography Unit (Avio TVS 2000) and Infrared Spot Thermometer (Minolta HT-11)

Prior to the commencement of the study, an initial investigation to correlate the infrared video thermography unit (Avio TVS 2000) and the infrared spot thermometer (Minolta HT-11) was carried out. Details of this study are given in Appendix 1. In summary, the two types of measuring devices were used to measure the temperature of post-mortem pig tissues at the same spot, as the tissues were exposed to therapeutic ultrasound. This was repeated several times as the tissue temperature changed with increased exposure. An intraclass correlation coefficient (single measure, two-way

mixed effect) was used to estimate the absolute agreement between the two measuring devices. The results show a correlation coefficient of 0.99 ($p<0.01$) for the two measuring devices, indicating an excellent agreement between the temperatures recorded by both devices.

3.4 GENERAL PROCEDURE

The general procedure common to all the studies and the selection of parameters for each individual study are summarized in Figure 3.1 (p86) and Table 3.1 (p87) respectively, and described in detailed as follows:

a. Preparation of specimens at abattoir (see Figure 3.1, item 1a, p86)

Arrangements were made with a local abattoir to supply the specimens. Only adult-sized pigs, weighing between 60 to 80 kilograms were chosen as specimens. The pigs were killed with a gunshot wound to the head, according to the rules and regulations of the Ministry of Agriculture, Forestry and Fisheries for the slaughter of animals meant for general consumption. Skin and hair were left intact, and thighs and shoulders were sectioned and placed in a storage room at 4°C until collection.

b. Collection and storage of specimens (see Figure 3.1, item 1b, p86)

Specimens were collected from the abattoir within 24 hours of slaughter. At the laboratory, they were stored in a refrigerator at 4°C. Under these conditions, the specimens could be stored for up to 10 days without spoiling. On the day of the experiments, the specimens were removed from refrigeration and placed at room temperature for about 4 to 6 hours to allow the tissue temperature to stabilise to its surroundings.

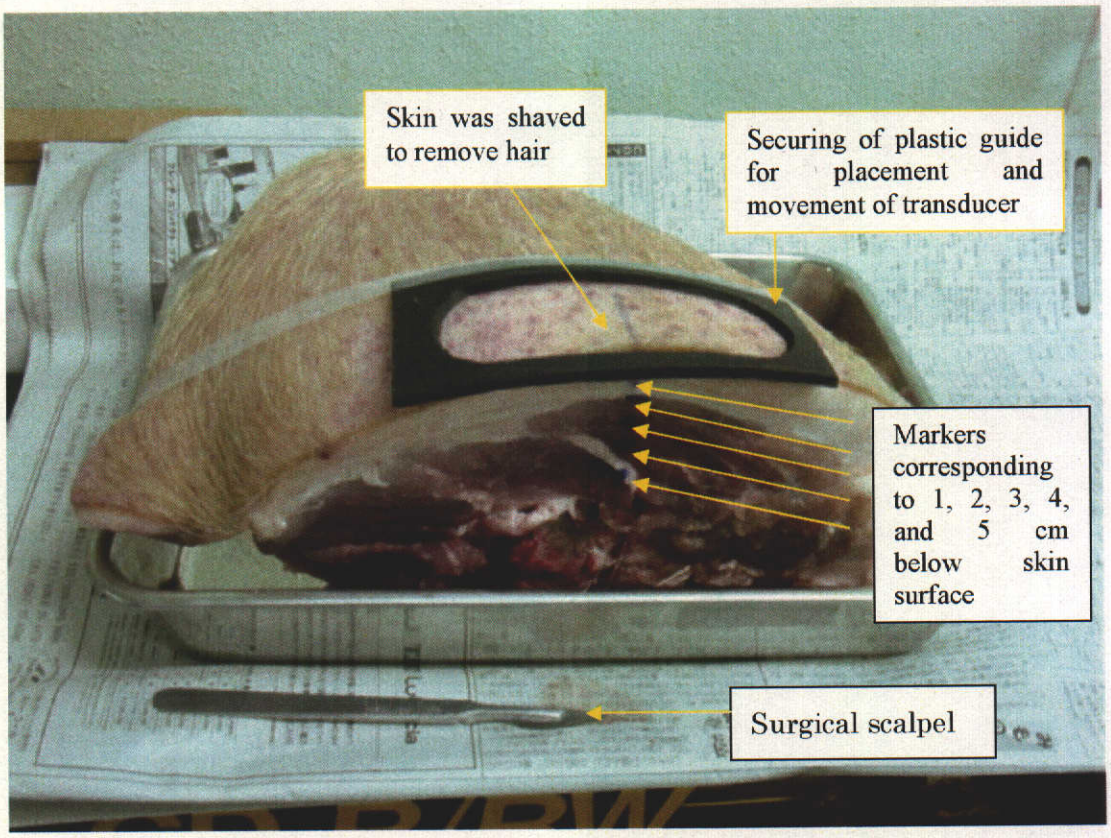

Figure 3.6: Preparation of the specimen for experimentation

c. Preparation of specimens at laboratory (see Figure 3.1, item 1c, p86)

At the laboratory, the specimens were then prepared as follows (see Figure 3.6):

① Removal of excess tissue with a surgical scalpel (no. 23 blade, no. 4 handle) on the main side to form a smooth and clean cross section. Only specimens with 1 to 2 cm of subcutaneous fat, and 7 to 8 cm of muscle tissue that was free from bone were used.

② Hair on the skin surface was shaved using a standard razor blade at the site where the experiments were to be conducted,

③ Markers corresponding to 1, 2, 3, 4 and 5 cm from the skin surface were made with an anatomical marker. These markers served as guides for subsequent identification of markers on the display unit of the infrared video thermographic camera, the Avio TVS 2000.

④ A thermoplastic guide corresponding to a specific size pre-determined for each study (see Table 3.1, p87) was secured to the shaved area of skin to delineate the tissue region to be exposed to the ultrasonic energy during the experiments.

d. Preparation of instruments at laboratory (see Figure 3.1, item 2a, p86)

The tissue specimen was placed on a stainless steel dissecting tray and set aside on one corner of the workbench. The ultrasound unit (Omnisound 3000) was placed next to the tissue specimen on the same workbench. The infrared thermographic camera was mounted on a standard camera tripod, adjusted to the height of the workbench and placed directly in front of the specimen, at a standard distance of 50 cm. The main processor unit of the thermographic camera was placed to the left of the ultrasound unit on the workbench. A television unit and video recorder were sited next to the main processor unit. The laboratory was in a sealed room with double doors and no windows, in order to prevent any wind drafts and mechanical vibrations. The room temperature of the laboratory was kept between 20 to 25°C by an air-conditioner. Only the investigator was in the room during the entire period of the data collection. All other sources of infrared radiation, such as coil heaters, were removed from the room in order to minimise potential noise artifacts, which could be picked up by the infrared thermography system.

e. Checking the output of the ultrasound machine (Figure 3.1, item 2b, p86)

The power meter, Model UPM-DT-10 by Ohmnic Instruments was used to check the output intensity and calibrate the Omnisound 3000™ ultrasound machine. The intensity output of the machine was checked prior to the commencement of experiments on every specimen. Calibration was only performed when the checks demonstrated an error in the output intensity of the machine exceeded ±10% (Figure 3.1, 2c, p86). On average, the Omnisound 3000™ required calibration at least once every fifth or sixth specimen. At all times the checking procedure was carried out in an enclosed room without any air currents or mechanical vibrations as follows:

① With the power meter positioned on a stable, level surface, the target cone was placed at the bottom of the test tank of the power meter. The test tank was then filled with de-gassed water (room temperature) to about 1 cm below the top of the rubber liner.

② Using the positioning clamps of the power meter, the transducer of the ultrasound unit was placed in the test tank with the radiating surface of the transducer about 1 cm below the water level in the test tank (see Figure 3.7, p96). The orientation of the transducer head for the Omnisound 3000 can be swiveled to be either parallel or perpendicular to the handle. For treatment purposes, the surface of the transducer was usually oriented parallel to the handle for easy manipulation. However, for calibration purpose, the surface of the transducer was oriented perpendicular to the handle as shown in Figure 3.7.

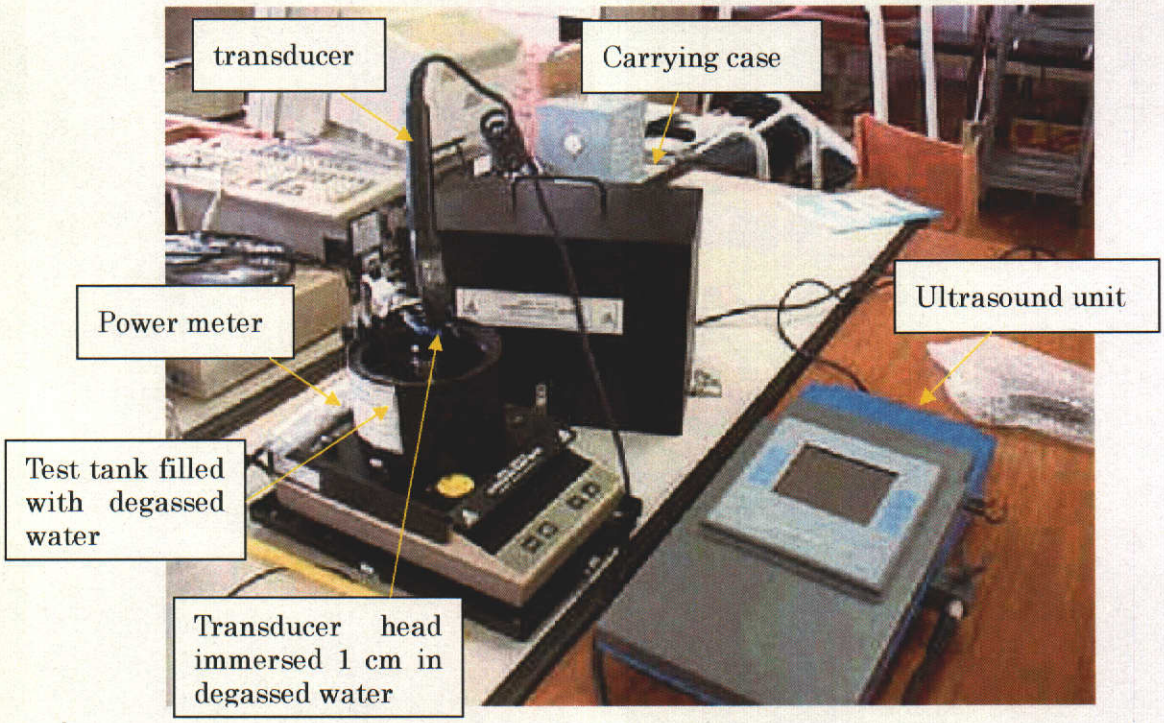

Figure 3.7: Calibration of the ultrasound unit using a power meter

③ The power meter was switched on and allowed to stabilise for 5 minutes. Following this, the power meter was re-set to zero, and the ultrasound unit was switched on and ready for checking or calibration.

④ The timer of the ultrasound machine was set to the maximum (20 minutes). Since adjustment of the output intensity button was only possible when the timer was set. As the checking procedure usually took about 10 to 15 minutes, the timer button did not need to be re-adjusted.

⑤ The output intensity was initially set at 1.0 Watts (0.1 Watts/cm^2) and the display on the power meter was checked to see if it corresponded appropriately (or within ±10%). The readings were recorded before increasing the output intensity from the ultrasound unit, and this procedure was repeated until the full range of output intensities for

Table 3.3: Range of intensities checked during calibration procedure.

1 MHz		3 MHz	
Watts/cm^2	Watts	Watts/cm^2	Watts
0.1	1.0	0.1	1.0
0.3	2.0	0.3	2.2
0.5	3.0	0.5	3.4
0.7	4.0	0.7	4.6
0.9	5.0	0.9	5.6
1.0	5.4	1.0	6.0
1.1	6.0	1.1	6.8
1.3	7.0	1.3	8.0
1.5	8.0	1.5	9.0
1.7	9.0		
1.9	10.0		
2.1	11.0		
2.3	12.0		

1 MHz and 3 MHz (see Table 3.3) were checked against the power meter readings as described. Upon completion, the recordings were re-checked to see if any of the readings were outside the 10% acceptable error range. If none were outside the error range, data collection was commenced. If any of the readings were outside the error range, the machine was calibrated and rechecked until all the intensities (Table 3.3) were within the ±10% error margin.

f. Calibration of ultrasound machine (see Figure 3.1, items 2c, 2d, p86)

The instrument setup for the calibration procedure was the same as for checking the intensity output. The calibration procedure for the Omnisound 3000^{TM} is a software-driven procedure in-built in the ultrasound machine. The purpose of the software is to adjust the display reading of the Omnisound 3000^{TM} to match the

display reading on the power meter. The step-by-step detail of the procedure is given in Appendix 2. In summary, it begins with calibration of the 1 MHz transducer at 4 points in the intensity range (12.0W, 9.0W, 5.0W, and 2.0W). This process is repeated for the 3 MHz transducer at 4 points in the intensity range (9.0W, 7.0W, 5.0W, 2.0W). By following the on-screen instructions, the ultrasound intensity display is adjusted to match the same intensity display on the power meter.

g. Commencement of separate studies (see Figure 3.1, item 3, p86)

There were a total of one pre-study on reliability, three studies on protocol-related issues, and one main study to determine the relationship between frequency, intensity and duration of exposure on depth of heating. The studies were carried out in the following sequence:

(1) Pre-study: Reliability of infra-red thermometry in measurement of temperature increases in post-mortem pig tissues exposed to therapeutic ultrasound (Chapter 4).

(2) Procedural Study 1: The effect of varying the movement speed (slow, moderate and fast) of the transducer on temperature increases in post-mortem pig tissues exposed to therapeutic ultrasound (Chapter 5).

(3) Procedural Study 2: The effect of varying the size of the treatment area (small, medium and large) on temperature increases in post-mortem pig tissues exposed to therapeutic ultrasound (Chapter 6)

(4) Procedural Study 3: A qualitative histological analysis of the effect of varying the output intensity on cell viability and thermal damage in post-mortem pig tissues exposed to therapeutic ultrasound (Chapter 7).

(5) Main Study: The effect of varying the frequency, output intensity and duration of exposure on increase in tissue temperatures at various depths in post-mortem pig tissues exposed to therapeutic ultrasound (Chapter 8).

h. Set-up of experimental procedure (see Figure 3.1, item 4, p86)

The prepared specimen was mounted on a fixed table, with the clean cross-section facing the infrared thermographic camera. Surface skin temperature was recorded with the infrared spot thermometer (Minolta HT-11) while the subcutaneous temperatures (1, 2, 3, 4 and 5 cm below skin) were measured with the infrared thermography unit (Avio TVS 2000). The camera to specimen distance was standardised at 50 cm for all experiments. Markers corresponding to 1, 2, 3, 4, and 5 cm on the specimen were plotted on the display unit, and saved to a 3.5 inch floppy disk. This served as a reference for subsequent data analysis for that particular experiment. Sensitivity of the camera and temperature ranges were set on the main-processor unit to allow for the measurement of the full range of temperature changes. The sensitivity was set at 0.04 to 0.10 and the temperature ranged from 15°C to 40°C or from 20°C to 45°C, depending on the specimen. The emissivity setting was standardised at 0.98 to correspond to human tissues. The video output from the main-processor unit was linked to a video tape recorder and television monitor, and the entire experiment was recorded on VHS tape. The VHS tape served as a backup in the event that any part of the experiment required clarification

or review. Measurements were recorded at baseline (prior to commencement of the experiment) and subsequently at 1-minute intervals during 10 minutes of exposure to the ultrasound, and for a further 10 minutes post-exposure, until the end of the experiment at 20 minutes. At the end of 20 minutes, the tissue specimen was left standing at room temperature to cool down for at least an hour. The temperatures at the measurement sites (surface, 1, 2, 3, 4 and 5 cm below surface) were monitored to ensure that the temperatures had returned sufficiently to baseline prior to commencement of the next experiment. In order to minimize the confounding effect of an eventual increase in starting temperatures between each individual experiment, the order of individual experiments for each study was randomized. All measurement data collected from the infrared thermography unit were saved to 3.5-inch floppy disks.

i. Selection of experimental parameters (see Figure 3.1, item 5, p86)

The experimental parameters specific to each study are summarized in Table 3.1 (p87). In general, there were five main parameters for all the studies: the movement speed of the transducer, the size of the treatment area, and the frequency, intensity and duration of exposure and post-exposure to ultrasound. These five parameters represented the independent variables for all the studies. The dependent variable throughout was change in tissue temperature (measured in ^{o}C) at the skin surface, and at 1, 2, 3, 4 and 5 cm below the skin surface.

j. Completion of experiments and disposal of specimens (Figure 3.1, item 6, p86)

On completion of each individual experiment, used specimens were stored frozen until all specimens from one animal had been used, at which time all samples

from that animal were returned to the abattoir for disposal. The abattoir disposed of waste tissue according to standard procedures, via on-site incineration.

3.5 DATA ANALYSIS

Data were analysed using the SPSS for Windows software, Version 10.0 (SPSS Inc., 444N Michigan Avenue, Chicago, Illinois 60611, USA). Analysis of the data was performed on change in temperature, rather than actual temperature measured at selected time points. Only data from the 5^{th}, 10^{th}, 15^{th}, and 20^{th} minutes were analysed. This corresponded to the middle and end of the ultrasound exposure phase (5^{th} and 10^{th} minute) and post-exposure phase (15^{th} and 20^{th} minute) as they were found to be representative of both these phases of data collection during the reliability study (Chapter 4). However, data for all 20-minute sampling are provided in the table of means for each experiment. The level of statistical significance was set at 0.05.

Specific details of the data analysis for each study are summarized in Table 3.1 (p87), and will be discussed in detail in Chapters 4 to 8.

3.6 ETHICAL CONSIDERATIONS

This study was reviewed by the Animal Experimentation Ethics Committee of Curtin University of Technology, Perth, Western Australia. As the animal tissues were obtained after the animals were killed at the abattoir, and disposal of used tissues was undertaken at the abattoir supplying the specimens, only a register number (E29/2000) was required and provided. According to the regulations of the Animal Experimentation Ethics Committee (AEEC), projects involving the use of tissues from animals deceased at the time of acquisition by the experimenters only

require notification to the AEEC in writing of the species, number and source of the animal tissue (email communication from Dr Norman Gare, Chairperson, AEEC dated 31 March 2000). This requirement was complied with during the period of the study.

Furthermore, since the data collection was carried out in Shinshu University, Japan, it was also necessary to ensure that the study complied with the ethical guidelines of that institution. For studies of this nature, involving use of tissues from animals deceased at the time of acquisition by the experimenters, current guidelines also did not require specific approval. Shinshu University guidelines required the investigators to ensure that the abattoir supplying the tissues abided by the rules and regulations of the Ministry of Agriculture, Forestry and Fisheries in the preparation and disposal of the tissues, as well as hygiene restrictions in the handling, storage and disposal of the tissues. All guidelines were strictly adhered to during the course of the study, as follows:

- The animals were killed by a gun-shot wound to the head, without the injection of any chemicals.
- The slaughtered animal was prepared separately from other slaughtered animals that were meant for human consumption. Preparation at this stage involved removal of the head, trotters, and all internal organs. The surplus tissues were disposed of by the abattoir via on-site incineration.
- The prepared specimens were stored separately at the abattoir prior to collection (within 24 hours) by the investigator.
- Upon collection, the specimens were stored in one of two large refrigerators dedicated exclusively for this study. After the experiments, the specimens were stored in a freezer, again dedicated exclusively for this study, until such time when they were returned to the abattoir for disposal via on-site incineration.

3.7 SUMMARY

The common features in the methodology and treatment parameters for each of the experiments have been summarized in Figure 3.1 (p86) and Table 3.1 (p87) respectively. The following chapters will describe the specific hypotheses and procedural details, together with the results of the individual investigations.

CHAPTER FOUR

RELIABILITY OF INFRA-RED THERMOMETRY IN MEASUREMENT OF TEMPERATURE INCREASES IN POST-MORTEM PIG TISSUES EXPOSED TO THERAPEUTIC ULTRASOUND

4.1 INTRODUCTION

Therapeutic ultrasound is a treatment modality that is widely used in the management of various medical conditions (McDiarmid and Burns 1987). Ultrasound is a form of mechanical wave motion, similar to audible sound, except that it is much higher in frequency (greater than 16 kHz) and inaudible to the human ear. As ultrasonic energy passes through the body's tissues, attenuation of the energy occurs due to absorption and scattering, with increased loss of energy as the tissue depth increases (ter Haar 1987). The ultrasonic energy that is absorbed by the tissues is converted to thermal energy, resulting in tissue heating (Wells 1977).

In order to examine the relationship between frequency, output intensity and duration of exposure to ultrasound on the heating pattern of tissues, accurate and precise measures of tissue heating are required. Various types of instruments have been used to measure increase in tissue temperatures exposed to therapeutic ultrasound. These measurement instruments include contact and non-contact temperature sensors such as thermistor probes and infrared thermometry. However, the use of infrared thermometry is relatively new (Armstrong 1997). Infrared thermometry includes spot (beam) thermometers as well as video thermography units.

Infrared thermometry has the unique advantage of offering access to the cross-sectional temperature profile of the tissues, as opposed to the conventional method of using thermistor probes that only provide temperature measurements at specific points. The reliability of infrared thermometry for measuring tissue temperatures has not been previously established.

The purpose of this study was to examine the within-session test-retest reliability of infrared thermometry, operated by a single investigator, to record and measure temperature changes at various distances from the skin surface in post-mortem pig tissues exposed to 1 MHz therapeutic ultrasound. Two types of infrared thermometry were assessed: the portable infrared spot (beam) thermometer (Minolta HT-11, Minolta Co. Ltd., Japan), and the infrared video thermography unit (Avio thermal video system TVS 2000™, Nippon Avionics Co. Ltd., Japan).

4.2 METHODS

a. Specimens

Thirteen tissue specimens, obtained from adult post-mortem pigs, were processed as previously described (Chapter 3, Section 3.4, pp92-94).

b. Instrumentation and Procedure

The instrumentation and general procedure have been described in Chapter 3 (sections 3.3 and 3.4, pp88-100). Therapeutic ultrasound at 1 MHz was applied to the tissues at 1.0 Watt/cm^2 for 10 minutes. The size of the treatment area was standardized at twice the size of the transducer, and was maintained using a thermoplastic guide, which was secured over the shaved skin of the designated treatment area. A direct in-contact moving soundhead technique using ultrasonic

gel was applied. The movement speed of the transducer was standardized at 120 beats/minute, maintained using a metronome.

For the initial test measurements, increases in tissue temperatures at six sites (skin surface, and 1, 2, 3, 4 and 5 cm below the skin surface) were recorded at baseline (prior to exposure) and subsequently at 1-minute intervals during the 10 minutes exposure, as well as for another 10 minutes post-exposure. Surface skin temperature was recorded with the infrared spot (beam) thermometer (Minolta HT-11) while the subcutaneous temperatures (1, 2, 3, 4 and 5 cm below skin surface) were measured with the infrared video thermography unit (Avio TVS 2000).

At the end of 20 minutes, the tissue specimen was left standing at room temperature to cool down for at least an hour (see p100). The experimental setup was left in situ. For the second test measurements, the experimental procedure was repeated using exactly the same parameters and procedure. The same investigator performed both the first and second test measurements.

At the end of the experiments, the specimens were disposed of according to the procedure described in Chapter 3 (section 3.4, paragraph j, p100)

c. Data analysis

A general description for data analysis has been provided in Chapter 3 (section 3.5, p101).

Intraclass correlation coefficients (single measure, two-way mixed effect model) were used to assess the within-session test-retest reliability of change in temperature for the 10 minutes exposure phase and 10 minutes post-exposure phase at the skin surface for the infrared spot thermometer, as well as at 5 tissue depths for the infrared video thermography unit.

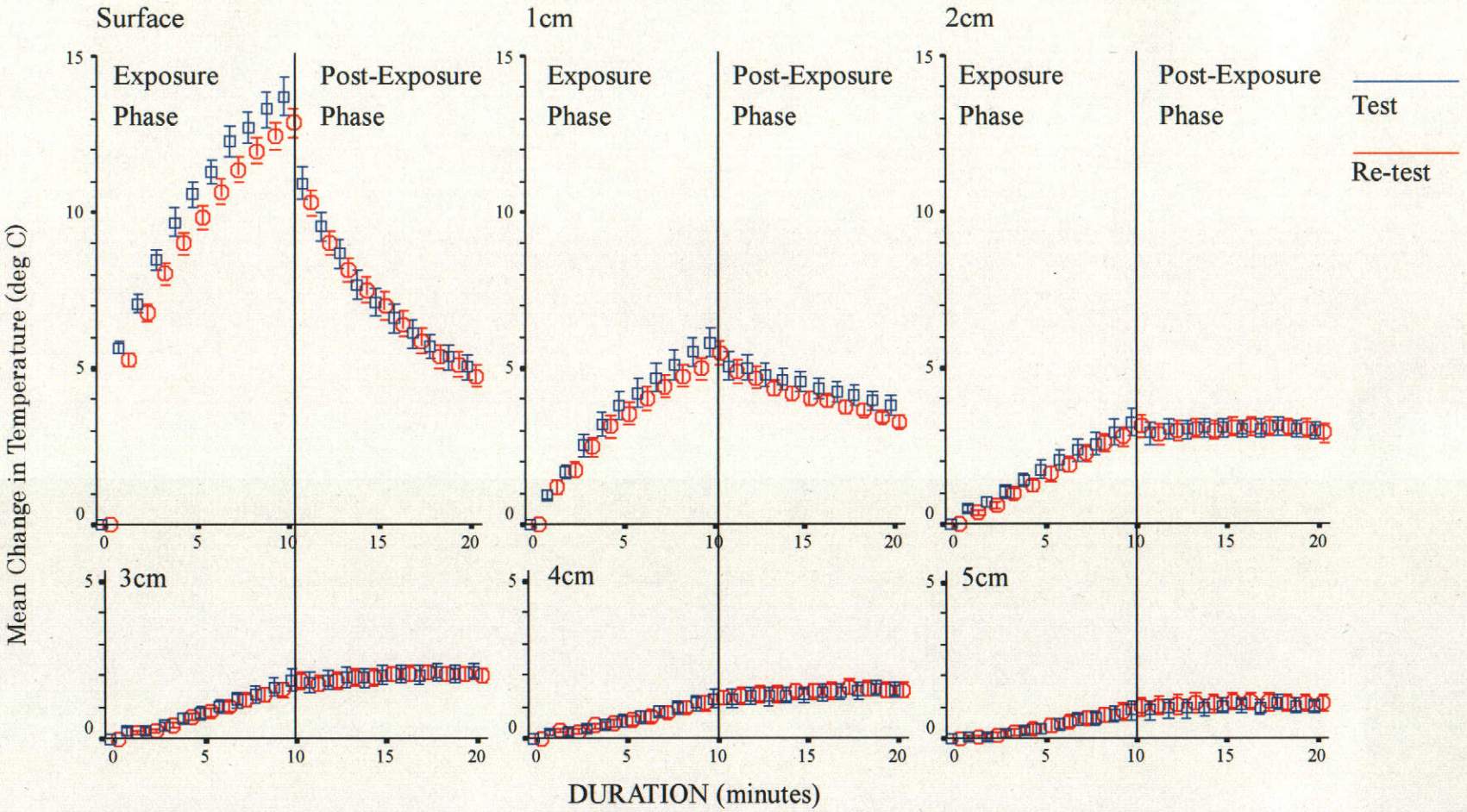

Figure 4.1: Mean (SE) change in temperature during exposure (1 to 10 minutes) and post-exposure (11 to 20 minutes) to 1MHz therapeutic ultrasound at 1.0 Watts/cm^2 for test and retest sessions at skin surface (top left), 1 cm (top centre) 2 cm (top right), 3 cm (bottom left), 4 cm (bottom centre), and 5 cm (bottom right) below the skin surface.

Table 4.1: Summary of intraclass correlation coefficients (ICCs) (two-way mixed effect model) for infrared spot thermometer (surface) and infrared video thermography unit (1, 2, 3, 4, 5 cm) for 1MHz ultrasound (1.0Watt/cm^2, 2X ERA, 120beats/min).

Duration	Surface	1cm	2cm	3cm	4cm	5cm
1	0.34	0.51	0.42	0.22	0.16	0.06
2	0.55	0.79	0.44	0.51	0.66	0.51
3	0.43	0.53	0.45	0.68	0.58	0.60
4	0.49	0.53	0.58	0.80	0.82	0.71
5	0.55	0.57	0.57	0.80	0.73	0.80
6	0.58	0.57	0.57	0.91	0.83	0.88
7	0.62	0.34	0.56	0.84	0.79	0.85
8	0.65	0.48	0.57	0.90	0.87	0.86
9	0.65	0.57	0.59	0.89	0.88	0.89
10	0.65	0.65	0.75	0.92	0.89	0.89
11	0.69	0.76	0.82	0.92	0.94	0.91
12	0.55	0.76	0.78	0.90	0.94	0.92
13	0.58	0.67	0.80	0.88	0.92	0.89
14	0.67	0.64	0.75	0.93	0.92	0.93
15	0.67	0.66	0.74	0.89	0.93	0.88
16	0.66	0.57	0.78	0.90	0.89	0.87
17	0.63	0.64	0.77	0.81	0.88	0.93
18	0.73	0.62	0.75	0.85	0.91	0.90
19	0.61	0.60	0.79	0.87	0.87	0.90
20	0.61	0.65	0.81	0.91	0.89	0.86
Miminum	0.34	0.34	0.42	0.22	0.16	0.06
Maximum	0.73	0.79	0.82	0.93	0.94	0.93

4.3 RESULTS

a. General

The change in temperature over time for test and retest sessions for the first 10 minutes exposure and subsequent 10 minutes post-exposure to ultrasound at the skin surface, and at 1, 2, 3, 4, and 5 cm below the skin surface is shown in Figure 4.1 (p107). The largest change in temperature occurred at the skin surface. As the distance from the surface increased, the change in temperature decreased (see Figure 4.1), and this was similar for both test and retest sessions. The change in temperature between test and retest sessions was almost identical at greater than 1 cm below the skin surface (see Figure 4.1).

A summary of the ICC scores for the infrared spot thermometer (surface) and the infrared video thermography unit (1, 2, 3, 4, and 5 cm below skin surface) is given in Table 4.1 (p108).

b. Infrared Spot Thermometer (skin surface)

The ICC scores for the infrared spot thermometer are given in Table 4.1 and summarized in Figure 4.2. The lowest and highest ICC scores were for the first (r=0.34) and 18th minute (r=0.73) respectively (Table 4.1). During the exposure phase, the ICC scores for the first six minutes were lower than 0.6 (Figure 4.2), and except for the second minute, the ICC scores increased as the duration increased from the first to the sixth minute. Furthermore, for the exposure phase, only the ICC scores for the 7th to 10th minutes were above 0.60 (Figure 4.2). In contrast, for the post-exposure phase, the ICC scores tended to be generally unchanged (Figure 4.2). The post-exposure phase was also generally more reliable than the exposure phase with most of their ICC scores (except for 12th and 13th minute) above 0.60.

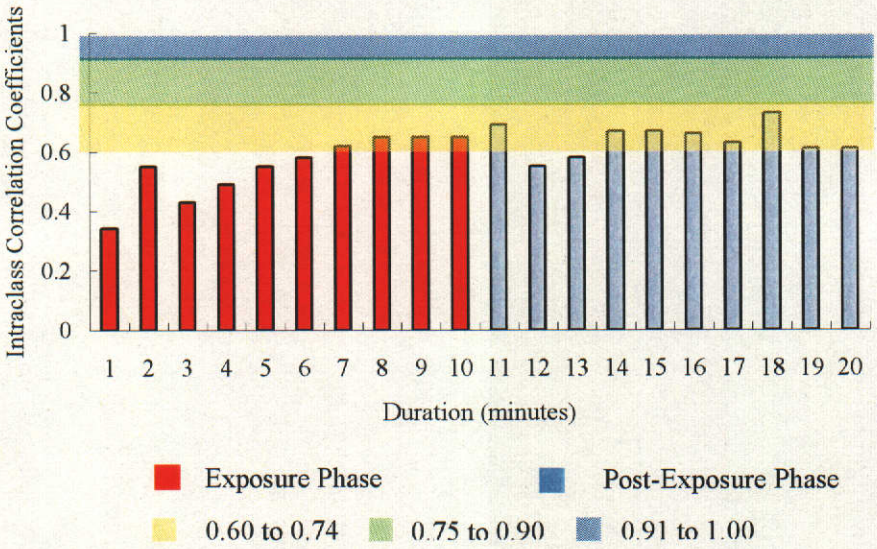

Figure 4.2: Summary of ICC scores for the exposure phase (1 to 10 minutes) and post-exposure phase (11 to 20 minutes) for the Infrared Spot Thermometer (Skin Surface).

c. Infrared Video Thermography Unit (1 to 5 cm below skin surface)

The ICC scores for the infrared video thermography unit are given in Table 4.1 and summarized in Figure 4.3 (exposure phase: top graph; post-exposure phase: bottom graph). The lowest and highest ICC scores were for the first minute at 5 cm (r=0.06), and 11th and 12th minutes at 4 cm (r=0.94) respectively (Table 4.1). In general the ICC scores for the first minute of the exposure phase were lower than the rest (Figure 4.3). During the exposure phase and post-exposure phase, the ICC scores increased as the depth of the target tissue increased, except for the first and second minute (Figure 4.3). The post-exposure phase was also generally more reliable than the exposure phase with most of the ICC scores for all tissue sites (except for 16th minute at 1 cm) above 0.60. In contrast, for the exposure phase, only the ICC scores for all tissue sites at the 10th minute were above 0.60 (Figure 4.3).

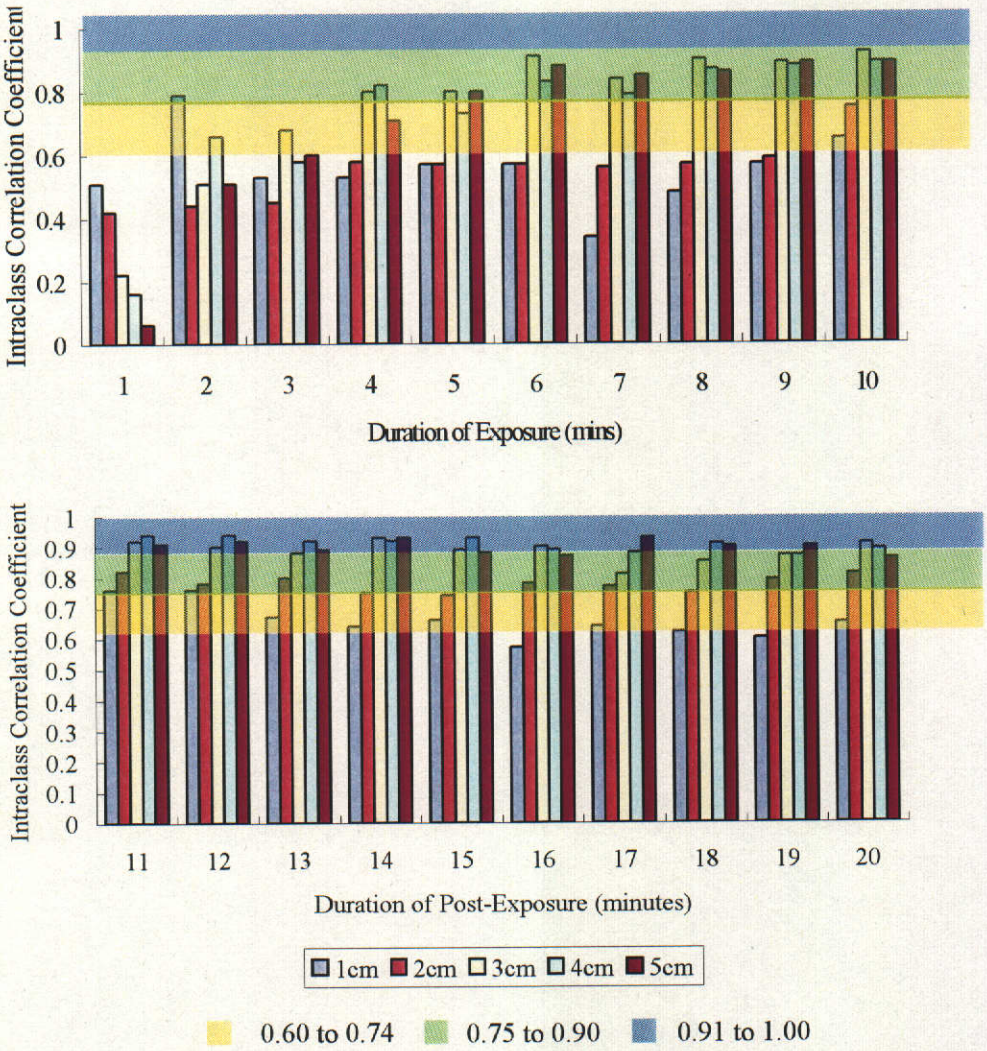

Figure 4.3: Summary of ICC scores for the exposure (1 to 10 minutes) phase (top) and post-exposure (11 to 20 minutes) phase (bottom) for the Infrared Video Thermography Unit (1 to 5 cm below skin surface)

d. Other results

The tables of means, standard deviations, single measure ICCs (two-way mixed effect model), 95% confidence intervals, F values, degrees of freedom, and p values for both the HT-11 infrared spot thermometer and the Avio TVS 2000 infrared video thermography unit are included in Appendix 3, Tables A3-1 to A3-6.

In addition, a summary of the intraclass correlation coefficients (ICCs) at 5 and 10 minutes (exposure phase), and 15 and 20 minutes (post-exposure phase), as

well as their statistical significance (p value) standard error of measurement (SEM) and 95% confidence intervals (CI) are given in Appendix 3, Table A3-7 for the HT-11 infrared spot thermometer (surface), and for the Avio TVS 2000 infrared video thermography unit (1, 2, 3, 4, 5 cm).

4.4 DISCUSSION

a. General

Reliability is defined as the consistency and dependability of a measure (Thomas and Nelson 1990), and test-retest reliability is best assessed by the ICCs (Ottenbacher and Stull 1993). However, there are no existing guidelines regarding what values are considered as "acceptable" or "good" reliability. Portney and Watkins' (1993) suggested that "how much" reliability is needed to justify the use of particular equipment must be determined by the researcher on a case-by-case basis. At the moment, there are no similar studies examining the reliability of the infrared spot thermometer and the infrared video thermography unit, and hence, comparable data is unavailable. However, based on Portney and Watkins (1993, p514) recommendations that an ICC below 0.60 is considered poor reliability, 0.60 to 0.74 is moderate reliability, 0.75 to 0.90 is good reliability, and above 0.90 is considered excellent reliability, it can be seen that the ICCs obtained from this study range from poor to excellent (Table 4.1). In Figures 4.2 and 4.3, a yellow, green and blue band across the figures indicate moderate, good and excellent reliability respectively. For the infrared spot thermometer, the ICC scores ranged from poor to moderate reliability (Table 4.1, Figure 4.2). For the infrared video thermography unit, the ICC scores ranged from poor to good reliability for the exposure phase, and moderate to excellent reliability for the post-exposure phase (Table 4.1, Figure 4.3).

Figure 4.1 illustrates the change in temperatures during the exposure phase

(1 to 10 minutes) and the post-exposure phase (11 to 20 minutes). As expected, the change in temperature increased as the duration of exposure increased, up to the final 10^{th} minute. This magnitude of increase was also related to the depth of the target tissue, with peak temperatures decreasing as the depth increased (Figure 4.1). For the post-exposure phase (11 to 20 minutes), however, the pattern was quite unexpected. There were two discernible patterns for the superficial tissues (surface, 1 and 2 cm below surface) and for the deep tissues (3, 4 and 5 cm below surface). At the skin surface and at 1 cm below surface, there was a progressive decrease of the temperatures to the 1^{st} minute and 5^{th} minute levels respectively. At 2 cm below surface, the temperature was maintained at around the peak temperature, and remained relatively constant throughout the entire post-exposure phase. For the deep tissues at the 3, 4 and 5 cm below surface, there was a gradual increase in temperature (less than 1°C) over the entire 10 minutes post-exposure phase.

b. Infrared Spot Thermometer (skin surface)

Firstly, the reliability of the infrared spot thermometer for measurements taken at the skin surface was less during the exposure phase, compared with the post-exposure phase (Figure 4.2). The main reason for this could be the difference in the measurement technique between the exposure and post-exposure phases. During the exposure phase, the presence of the gel, as well as the direct contact between the skin surface and the ultrasonic transducer (which itself was being heated and getting progressively warmer during the application) could have been two factors that influenced the reliability of the measurements taken with the infrared spot thermometer. In addition, at every one-minute interval, the ultrasonic transducer was removed from the skin surface temporarily for about 3 seconds each time to allow the measurements with the infrared spot thermometer at the skin

surface to be taken without any obstruction. In contrast, during the post-exposure phase, the ultrasonic transducer was no longer in contact with the specimen. Apart from a thin layer of gel that was left in-situ at the end of the exposure phase, there was minimal interference during measurements taken at the skin surface during the post-exposure phase. This minimal interference at the site of temperature measurements could have contributed to the stability of ICC scores from the 11 to the 20^{th} minute (Figure 4.2). Another factor that could have affected the reliability of the exposure phase is the consistency of the output intensity from the ultrasound transducer. While every effort was made to calibrate and check that the error of the output intensity of the transducer prior to the experiments were always within $\pm$ 10%, it cannot be assumed that the intensity output was exactly the same for each ultrasound application. This slight variation in output intensity itself could have affected the reliability of the exposure phase. In contrast, the reliability of the post-exposure phase was not affected by any variation in the output intensity, as the transducer was no longer in contact with the specimens.

Secondly, the results demonstrate that during the exposure phase, there was a gradual increase in ICC scores from the first to the sixth minute, before stabilizing from the seventh to the tenth minute (Figure 4.2). The presence of the ultrasonic gel on the skin surface could have been the main cause, due to the initial mismatch of temperatures between the gel and the skin surface. As indicated by the gradual increase in ICC scores from the first to the sixth minute (Figure 4.2), the reliability improved with time as the mismatch of temperatures between the gel and the skin surface decreased with progressive heating of both substances. After the seventh minute, and presumably after the difference in temperatures between the two substances diminished considerably, the ICC scores appeared to stabilize and were generally above 0.60.

Thirdly, the reliability of the infrared spot thermometer at the skin surface for both the exposure and post-exposure phases were generally not as high as those of the infrared video thermography unit (Figure 4.2 and 4.3). The SEM scores were less than 1.5°C for the HT-11 infrared spot thermometer, and 1.05°C for the Avio TVS 2000 infrared video thermography unit (Appendix 3, Table A3-7), indicating that the latter was more precise than the former. While the poorer than expected ICC scores for the infrared spot thermometer during the exposure phase can be explained by the interference of the ultrasonic gel and direct contact with the ultrasonic transducer (see above), the same cannot be said for the post-exposure phase. Apart from the thin layer of gel left in-situ on the skin surface during the post-exposure phase, the measurement procedures for both infrared spot thermometer and the infrared video thermography unit were quite similar. Nevertheless, the results demonstrated that the reliability of the infrared spot thermometer was generally lower than the infrared video thermography unit during both the exposure and post-exposure phases (see Figure 4.2 and 4.3). Therefore, it appears the infrared spot thermometer is less reliable than the infrared video thermography unit possibly because of differences in the inherent accuracy and reliability of the electronic componentry.

c. Infrared Video Thermography Unit (1 to 5 cm below skin surface)

For the infrared video thermography unit, the cross-sectional surface of the specimen was exposed to the camera of the thermography unit, and measurements were taken with little or no interference during the entire exposure and post-exposure phases.

Firstly, the ICC scores for the first minute at all five tissue sites were the lowest compared with the other time intervals (see Figure 4.3). Prior to the

experiments, the specimens were stored in a refrigerator at 4°C. On the day of the experiments, the specimens were removed from refrigeration and placed at room temperature for about 4 to 6 hours to allow the tissue temperature to stabilize to its surroundings. However, the temperature of the tissue specimens was always below the room temperature at the commencement of the experiments. Initial measurements were taken while the tissue specimens were exposed to the ultrasound for 10 minutes, followed by post-exposure of another 10 minutes. The tissue specimens were then left in-situ for about one hour to allow for sufficient cooling before the commencement of the second measurements. While every effort was made to allow the tissues to cool down sufficiently, it was not possible to start the second set of experiments at exactly the same baseline temperature as the first measurements. Also, with time, the tissue specimens generally became warmer. This initial mismatch in starting temperatures, particularly within the first minute, could have contributed to the lower than expected ICC scores for the first minute at all tissue sites. This mismatch was greatest within the tissues, rather than at the surface. After the first minute, however, the mismatch in temperatures between the two measurements was not as great, and hence, the effect of this mismatch on the reliability of the measurements was reduced although not completely eliminated until around the 5^{th} or 6^{th} minute (Figure 4.3).

Secondly, the ICC scores for the exposure phase were generally lower than those for the post-exposure phase. While this can be partly explained by the mismatch in initial starting temperatures between the first and second measurements (see above), it is possible that other reasons could have also accounted for this difference. As in the infrared spot thermometer for measurements of the surface skin temperature, the amount of heating that reaches the target tissues during the exposure phase depended on the consistency of the output intensity of the ultrasound

transducer. The slight variance in the output intensity, albeit within $\pm 10\%$, was possibly the main factor that affected the reliability of both the infrared spot thermometer as well as the infrared video thermography unit during the exposure phase. The post-exposure phase, however, was probably dependent on the final increase in temperature just prior to cooling, more than any other factor. Hence, the reliability of the post-exposure phase would have been unaffected by the slight variance in output intensity and could be expected to be generally higher than the exposure phase, as was demonstrated in this study.

Thirdly, the ICC scores for the superficial tissues were generally lower than those for the deeper tissues, particularly at 1 and 2 cm below the skin surface. While the contact between the transducer and the skin surface was maintained by slight pressure from the weight of the transducer itself, a certain amount of tissue deformation, particularly near the surface, could not be avoided. This deformation of the superficial tissues was probably one of the main factors that resulted in the low ICC scores obtained at 1 and 2 cm below the skin surface. For the deeper tissue sites at 3, 4 and 5 cm below the skin surface however, no such tissue deformation occurred. As a result, the ICC scores for these three tissue sites were generally quite similar and consistently higher compared to the superficial tissues. Another possible reason was the difference in tissue types between the superficial and deep tissues. The tissue specimens were always carefully selected to ensure that the layer of fat below the skin surface was less than 2 cm. While there was a slight variation in the amount of fat-muscle tissue composition between each specimen up to 2 cm below the skin surface, little variation occurred at the deeper tissues (3, 4, and 5 cm). In other words, the tissues up to 2 cm depth were not always homogenous (fat-muscle composition), whereas the tissues at greater than 3 cm can be considered to have been always homogenous (muscle only). This

difference in the homogeneity between the tissues at the superficial and deep sites could have contributed to the poorer reliability in the former.

d. Other results

The main purpose of the study was to examine the test-retest reliability of the infrared spot thermometer (at the skin surface) and the infrared video thermography unit (from 1 to 5 cm below the skin surface). However, in order to simplify the data analysis for the duration factor (20 minutes) for subsequent studies, and to reduce the levels from 20 to a more manageable level, it was necessary to identify specific periods in the 20-minute duration, which could be considered representative of the exposure and post-exposure phases. These specific periods were identified by the good test-retest reliability as indicated by the high ICCs scores. In order to facilitate the identification of these "representative" time periods, the exposure and post-exposure phases were divided into four time frames: 1 to 5, 6 to 10, 11 to 15 and 16 to 20 minutes.

From Appendix 3, Table A3-1 to A3-6, it can be seen that for the exposure to ultrasound phase, the ICCs for the fifth minute were usually the highest for the first five minutes of exposure (ICCs ranged from 0.55 to 0.80). Furthermore, the ICCs for the 10^{th} minute were also usually the highest for the second five minutes of exposure (ICCs ranged from 0.65 to 0.92). For the post-exposure phase, although the ICCs for the 15^{th} minute were not necessarily the highest for the 11^{th} to 15^{th} minute period, nevertheless the ICCs for the 15^{th} minute can be considered to be reasonably high (ICCs ranged from 0.66 to 0.93). The ICCs for the 20^{th} minute were usually the highest for the 16^{th} to 20^{th} minute duration. Based on this observed trend, data from the 5^{th}, 10^{th} and 20^{th} minute could be considered most reliable because of the consistently high ICCs obtained within these specific time

periods. While data from the 15th minute were not consistently the highest within the time period (11th to 15th minute), they were always within the limits of acceptable reliability, as defined in this study. Hence, these four time periods could be considered representative of the exposure and post-exposure phases. Analyses of subsequent studies, therefore, could be simplified by confining it to the 5th, 10th, 15th, and 20th minutes of data only. The entire 20 minutes of data, however, will still be presented in the appendices of the relevant studies.

4.5 CONCLUSION

The results of this study demonstrate that for the procedure used, both the infrared spot thermometer and the infrared video thermography unit can be considered to be adequately reliable, with the latter demonstrating slightly better reliability than the former. In addition, because of the consistently high ICCs obtained for the 5th, 10th, 15th and 20th minutes, these data points can be considered representative of the exposure and post-exposure phases. This will reduce the number of levels for the duration factor from 20 to four, and thereby simplifying the data analyses considerably for subsequent studies. The results of this study also demonstrate that under the procedure and conditions specified, both the infrared spot thermometer (Minolta HT-11) as well as the infrared video thermography unit (Avio TVS 2000) are capable of recording temperature increases in tissues exposed to therapeutic ultrasound at an acceptable level of test-retest reliability, as defined by this study.

CHAPTER FIVE

THE EFFECT OF VARYING THE MOVEMENT SPEED (SLOW, MODERATE AND FAST) OF THE TRANSDUCER ON TEMPERATURE INCREASES IN POST-MORTEM PIG TISSUES EXPOSED TO THERAPEUTIC ULTRASOUND

5.1 INTRODUCTION

The "Guide Lines for the Safe Use of Ultrasound Therapy Equipment" (Chartered Society of Physiotherapy 1990) states that "the transducer should be moved slowly and continuously throughout treatment", possibly to reduce the sensation of hot spots under the transducer, as well as the formation of standing waves which can arrest blood flow (Low and Reed 2000). Hence, therapists usually move the transducer in either a circular fashion or a linear stroking manner, at a fixed or variable rate. In the literature review section in Chapter 2 (section 2.4.2, paragraph f, p35), it has been suggested that the movement speed of the transducer could possibly affect the consistency of the temperature increases in tissues exposed to therapeutic ultrasound. While the main aim of this thesis was to examine the relationship between the frequency, the output intensity and the duration of exposure to therapeutic ultrasound on increase in temperature at various distances from the skin surface, it was unclear whether the movement speed of the transducer could be a

confounding factor as there have been no studies to date that examined this issue. The purpose of this study was to investigate the effect of varying the movement speed of the transducer on temperature increases at various distances from the skin surface in post-mortem pig tissues exposed to therapeutic ultrasound.

5.2 NULL HYPOTHESES

The null hypotheses for this study were:

1. Varying the movement speed of the transducer (slow, moderate or fast) does not affect the temperature increases in post-mortem pig tissues exposed to a **1 MHz** frequency therapeutic ultrasound at:
   a. the skin surface,
   b. 1 cm below the skin surface,
   c. 2 cm below the skin surface,
   d. 3 cm below the skin surface,
   e. 4 cm below the skin surface,
   f. 5 cm below the skin surface.
2. Varying the movement speed of the transducer (slow, moderate or fast) does not affect the temperature increases in post-mortem pig tissues exposed to a **3 MHz** frequency therapeutic ultrasound at:
   a. the skin surface,
   b. 1 cm below the skin surface,
   c. 2 cm below the skin surface,
   d. 3 cm below the skin surface,
   e. 4 cm below the skin surface,
   f. 5 cm below the skin surface.

5.3 METHODS

a. Specimens

Twenty tissue specimens, obtained from post-mortem adult domestic pigs, were processed as previously described (Chapter 3, section 3.4, pp92-94). This experiment entailed testing at 2 frequencies and 3 movement speeds. Ten specimens were used for 1 MHz applications and the other 10 (matching contralateral side) for 3 MHz applications. Each specimen was used three times, once at each of the three movement speeds being tested in this study. Specimens were left for at least an hour at room temperature between applications to allow them to return to baseline temperature.

b. Instrumentation and Procedure

The instrumentation and general procedure have been described in Chapter 3 (sections 3.3 and 3.4, pp88-100). Therapeutic ultrasound at 1 MHz and 3 MHz frequencies was applied to the tissues at 1.0 Watt/cm^2 for 10 minutes. The size of the treatment area was standardized at twice the size of the transducer, and was maintained using a thermoplastic guide, that was secured over the shaved skin of the designated treatment area. A direct in-contact moving soundhead technique using ultrasonic gel was applied. Three transducer movement speeds were investigated; slow (60 beats/minute), moderate (120 beats/minute) and fast (180 beats/minute). For an area corresponding to 2X ERA with an overall diameter of 7cm, the movement speeds for slow, moderate and fast can also be described as 7cm/s, 14cm/s, and 21cm/s respectively. Movement speeds were maintained using a metronome.

The order of selection of the three movement speeds of the transducer was randomized. For the first speed selected, increases in tissue temperatures at six sites (skin surface, and 1, 2, 3, 4 and 5 cm below the skin surface) were recorded at baseline (prior to exposure) and subsequently at one-minute intervals during the

10-minutes exposure, as well as for another 10 minutes post-exposure. Surface skin temperature was recorded with the infrared spot thermometer (Minolta HT-11) while the subcutaneous temperatures (1, 2, 3, 4 and 5 cm below skin surface) were measured with the infrared video thermography unit (Avio TVS 2000). The test-retest reliability of the infrared spot thermometer and the infrared video thermography unit had been previously established (see Chapter 4).

At the end of 20 minutes, the tissue specimen was left standing at room temperature to cool down for at least an hour. The experimental setup was left in situ. For measurements of the second and third movement speeds of the transducer, the above experimental procedure was repeated using exactly the same parameters and procedure as before. In this manner, all experiments for all three speeds were performed on one tissue specimen. Experiments were repeated in the same manner for the second frequency, on a matching contralateral side. The same investigator performed all the measurements. The general procedure is summarized in Figure 3.1 (p86). The specific procedure for this study is summarized in Figure 5.1 (p123).

At the end of the experiments, the specimens were disposed of according to the procedure described in Chapter 3 (section 3.4, paragraph j, p100).

c. Data analysis

A general description for data analysis has been provided in Chapter 3 (section 3.5, p101). Data from all specimens were analysed; 10 specimens for 1 MHz and 10 specimens for 3 MHz. Analysis of the data was performed on change in temperature (dependent variable), rather than actual temperature measured, at the 5^{th}, 10^{th}, 15^{th}, and 20^{th} minutes. The reliability at these four time periods was found to be representative of the exposure and the post-exposure phases (Chapter 4).

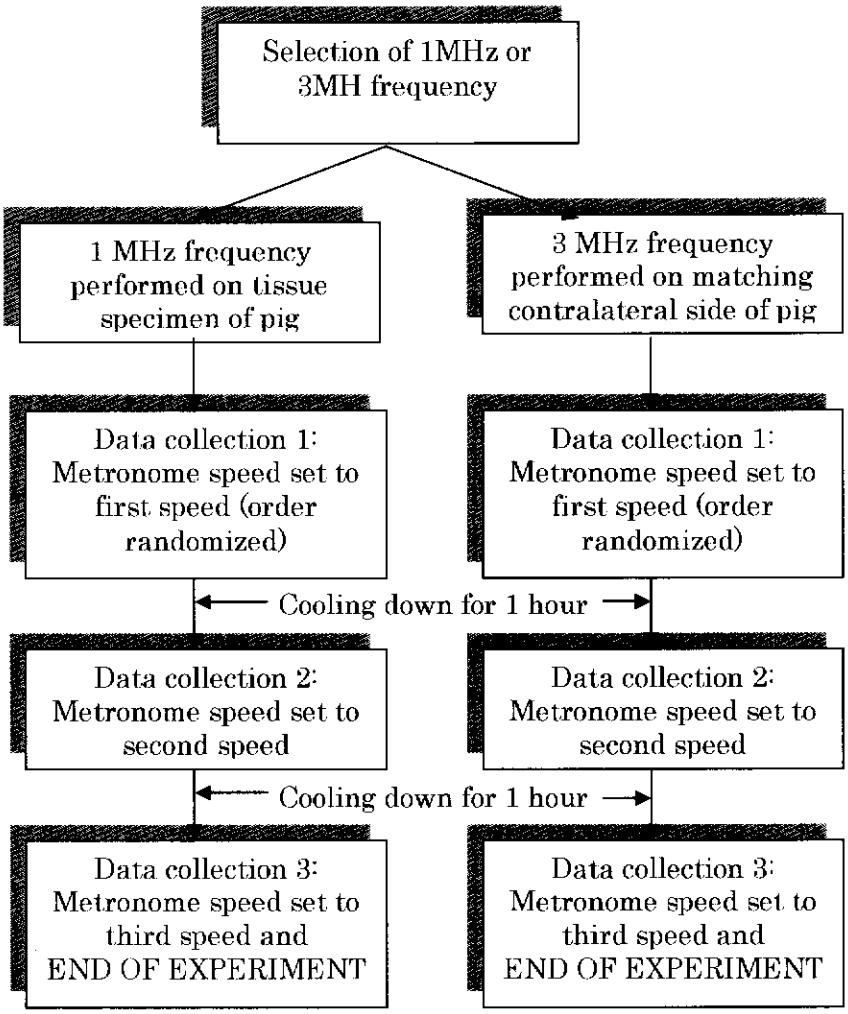

Figure 5.1: Flow chart of specific procedure

However, data for all 20-minute sampling are provided in the tables of means (Appendix 4) and in Figures 5.2 and 5.3.

A three (speeds – 60, 120, 180 beats/min) by two (frequencies – 1 and 3 MHz)) by six (sites – skin surface, and 1, 2, 3, 4, 5 cm below skin surface) by four (duration – 5, 10, 15 and 20 minutes) repeated measures analysis of variance was used to assess the effect of varying the speed on the change in tissue temperatures (dependent variable). For those factors that were found to be significantly different, a repeated contrasts (within subjects) post-hoc analysis on factors that had greater than two levels was performed to identify differences among the levels.

In addition, the observed power, the estimates of effect size and descriptive statistics for means and standard deviations of the scores were also calculated. The assumptions of sphericity, equal variance and normality of the statistical procedures were checked.

5.4 RESULTS

The main factor of interest in the analysis was speed and its interactions. A general description of the results will be presented first. Following this, the details of these results for speed, frequency, site and duration will be presented for their four-way interactions, followed by their three-way and two-way interactions, and finally for their main effects.

a. General

Figures 5.2 (for 1 MHz) and 5.3 (for 3 MHz) summarizes the change in temperature over time for slow, moderate and fast movement speeds of the transducer at the skin surface, and at 1, 2, 3, 4 and 5 cm below the skin. The largest change in temperature occurred at the skin surface for both 1 and 3 MHz and this was almost twice as great for 3 MHz (27.11°C at 10 minutes) as for 1 MHz (15.16°C at 10 minutes) (see Figures 5.2 and 5.3, and Table A4-1 in Appendix 4). As the distance from the surface increased, the change in temperature decreased, and this was similar for both 1 and 3 MHz. The change in temperature between slow, moderate and fast movement speeds of the transducer was almost identical at greater than 1 cm below the skin surface (see Figures 5.2 and 5.3).

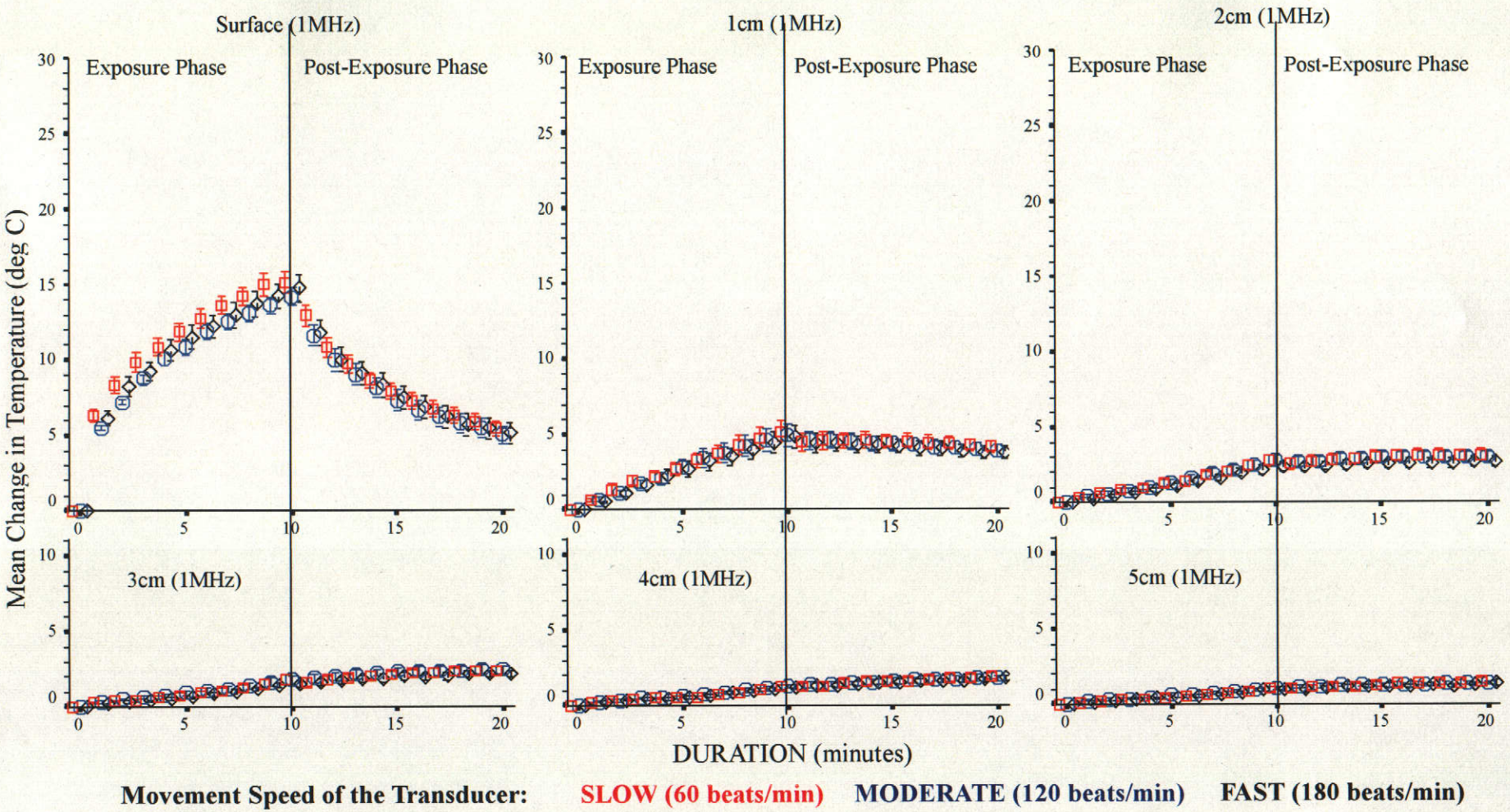

Figure 5.2: Mean (SE) change in temperatures for 10 minutes exposure and 10 minutes post-exposure to therapeutic ultrasound (x-axis) for 1MHz at 1.0 Watts/cm^2 at slow (60 beats/min), moderate (120 beats/min) and fast (180 beats/min) movement speed of the transducer

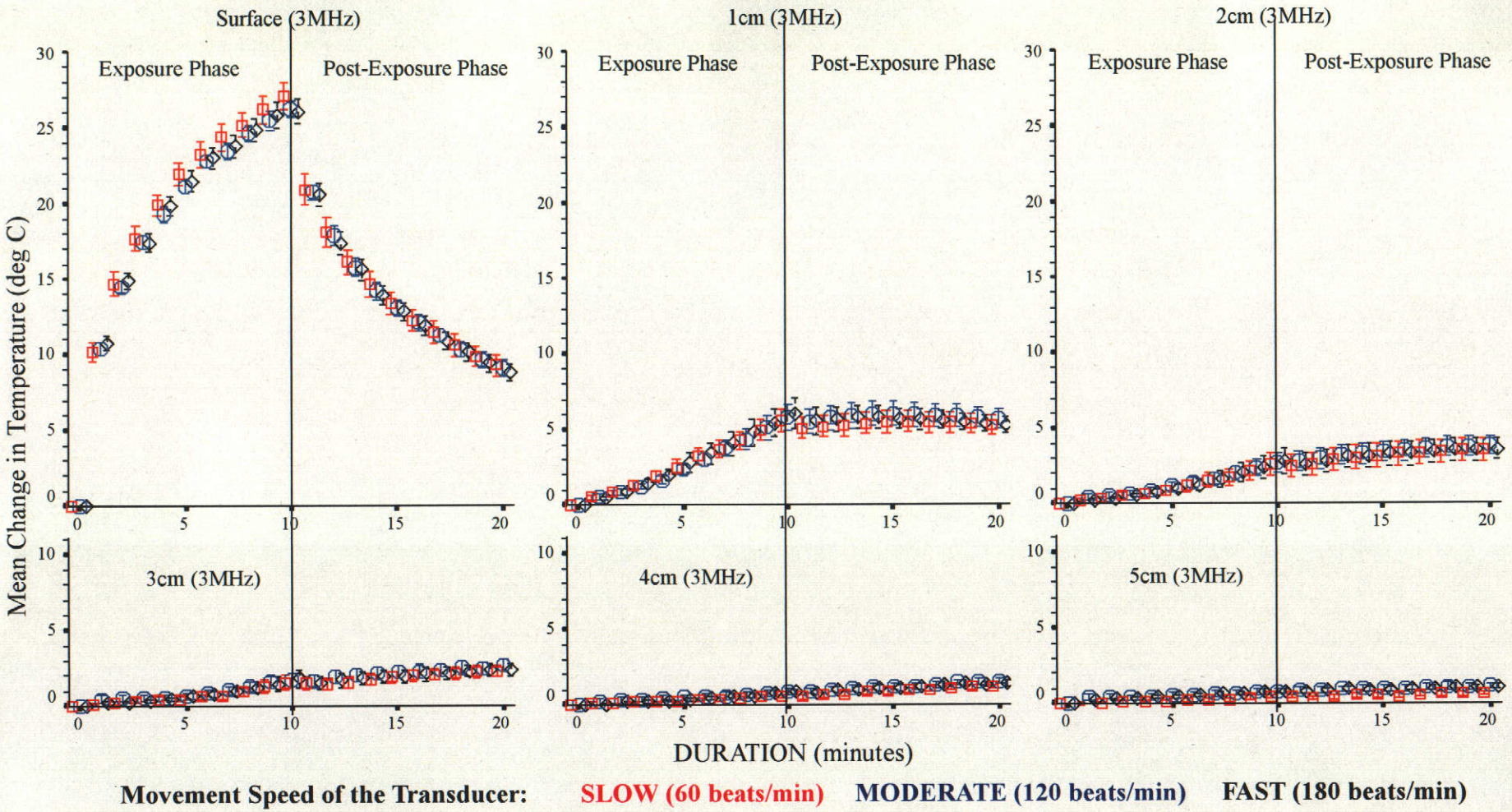

Figure 5.3: Mean (SE) change in temperatures (y-axis) for 10 minutes exposure and 10 minutes post-exposure to therapeutic ultrasound (x-axis) for 3MHz at 1.0 Watts/cm^2 at slow (60 beats/min), moderate (120 beats/min) and fast (180 beats/min) movement speed of the transducer

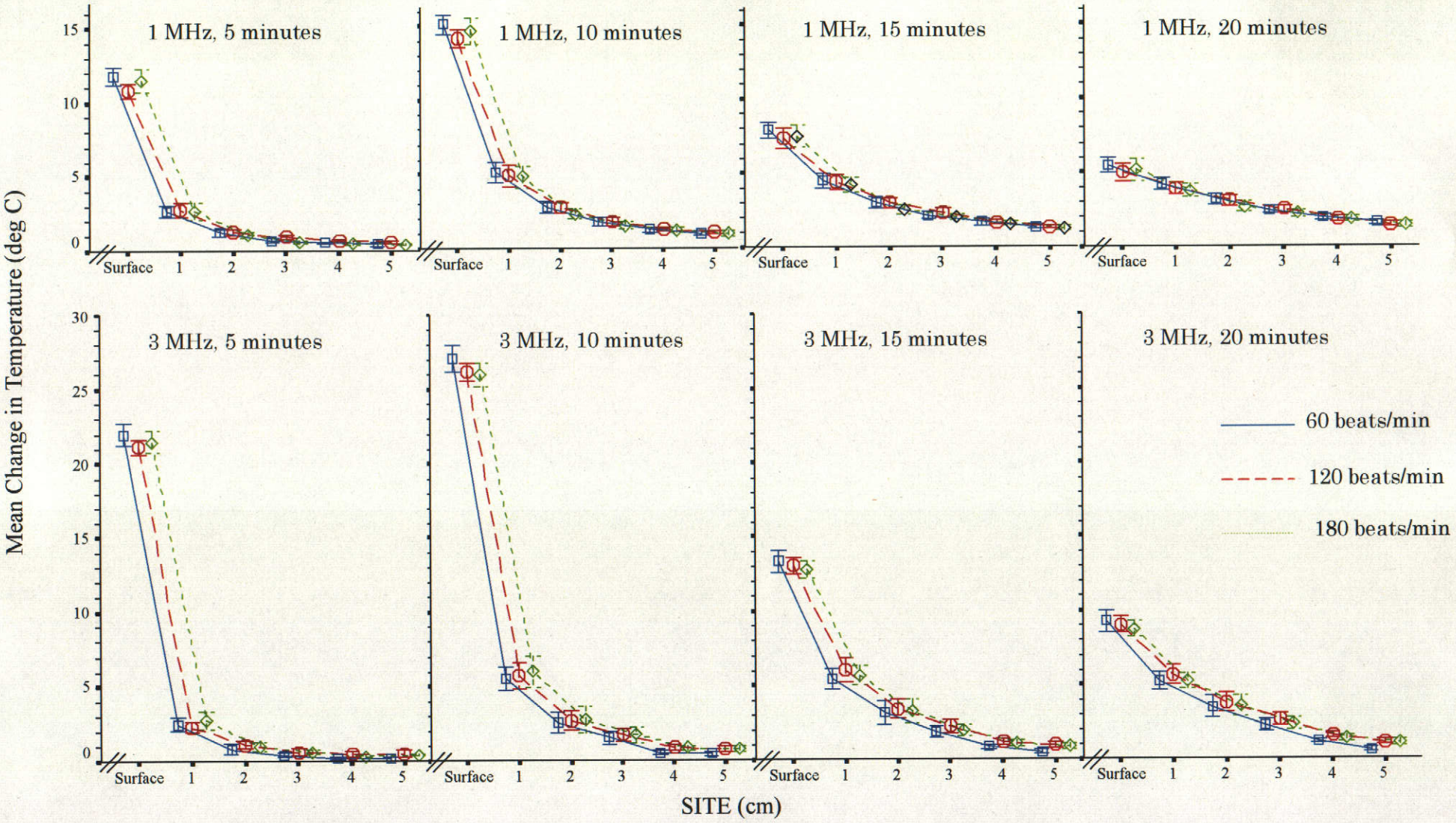

Figure 5.4: Mean (SE) change in temperature for four-way interactions among speed (slow – 60beats/min, moderate – 120beats/min, fast – 180beats/min), frequency (1, 3 MHz), site (surface, 1, 2, 3, 4, 5 cm) and duration (5, 10, 15, 20 mins).

Table 5.1: Repeated Measures ANOVA (General Linear Model, within subjects effects), and Contrasts (within subjects, repeated) for the four-way and three-way interaction effects on main factors (Frequency: 1, 3 MHz; Speed: 60, 120, 180 bpm; Duration: 5, 10, 15, 20 mins; Site: 0 or surface, 1, 2, 3, 4, 5 cm).

Factor				df	F value	P value	Eta	Power
Duration	Freq	Speed	Site	30	0.732	0.85	0.075	0.692
	Freq	Speed	Site	10	0.453	0.92	0.048	0.221
Duration		Speed	Site	30	1.318	0.13	0.128	0.957
Duration	**Freq**		**Site**	15	69.740	<0.01	0.886	1.000
5 vs 10	**1 vs 3**		5 vs 4	1	0.037	0.85	0.004	0.053
			4 vs 3	1	1.514	0.25	0.144	0.197
			3 vs 2	1	0.648	0.44	0.067	0.112
			2 vs 1	1	9.374	0.01	0.510	0.778
			1 vs 0	1	2.127	0.18	0.191	0.257
10 vs 15	**1 vs 3**		5 vs 4	1	1.760	0.22	0.164	0.221
			4 vs 3	1	0.039	0.85	0.004	0.054
			3 vs 2	1	7.103	0.03	0.441	0.661
			2 vs 1	1	0.206	0.66	0.022	0.069
			1 vs 0	1	236.742	<0.01	0.963	1.000
15 vs 20	**1 vs 3**		5 vs 4	1	2.531	0.15	0.220	0.296
			4 vs 3	1	5.435	0.05	0.377	0.548
			3 vs 2	1	0.199	0.67	0.022	0.069
			2 vs 1	1	0.908	0.37	0.092	0.137
			1 vs 0	1	48.555	<0.01	0.844	1.000
Duration	Freq	Speed		6	0.803	0.57	0.082	0.290

Freq = Frequency; df = degrees of freedom; Eta = Estimated effect size; bpm = beats per minute. **(Note: Bold fonts indicate significant effects or interactions)**

b. Results of analysis for four-way interactions

(1) Speed, Frequency, Site and Duration

The results demonstrated that there was no significant 4-way interaction among speed, frequency, site and duration on the change in temperature ($F_{30, 270}$=0.732, p=0.85; Figure 5.4, Table 5.1). As frequency and duration of exposure increased, and as distance from the surface and duration of post-exposure decreased, the change in temperature was greater regardless of the movement speed of the transducer.

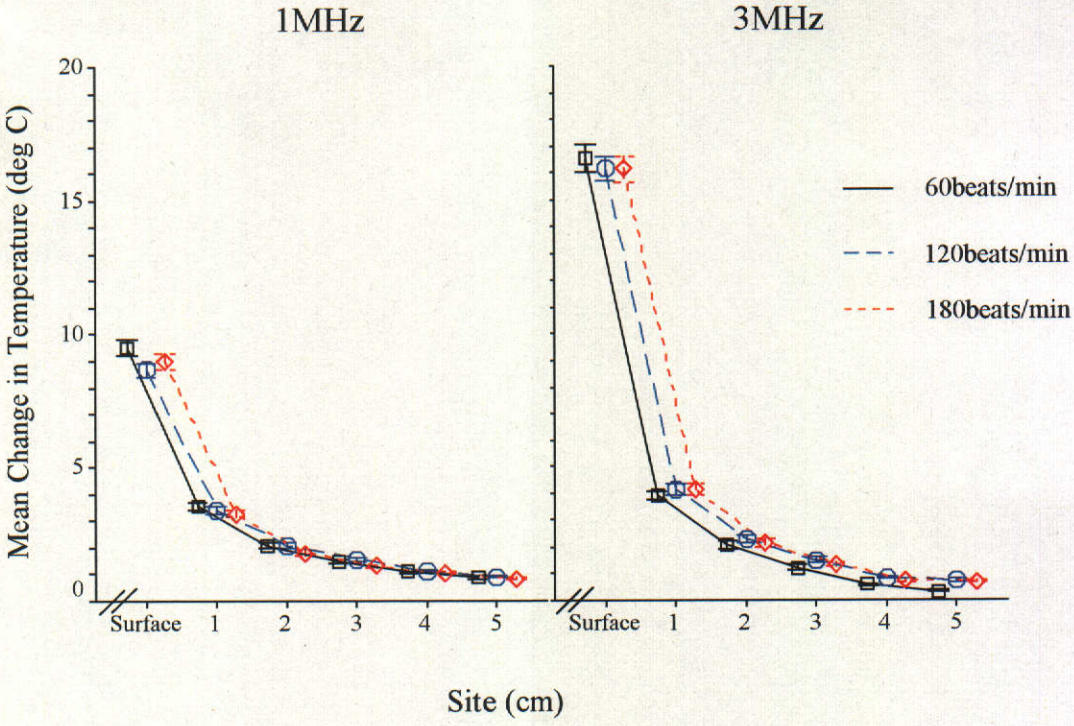

Figure 5.5: Mean (SE) change in temperature for three-way interactions among speed (slow – 60beats/min, moderate – 120beats/min, fast – 180beats/min), frequency (1, 3 MHz) and site (surface, 1, 2, 3, 4, 5 cm).

c. Results of analysis for three-way interactions

(1) Speed, Frequency and Site

The results demonstrated that there was no significant 3-way interaction among speed, frequency and site on the change in temperature ($F_{10, 90}$=0.453, p=0.92; Figure 5.5, Table 5.1). Regardless of the movement speed of the transducer, the change in temperature was greater at the surface for the 3 MHz frequency compared with the 1 MHz frequency. However, at the other tissue sites, the changes in temperature were not influenced by the movement speed or the frequency of the transducer.

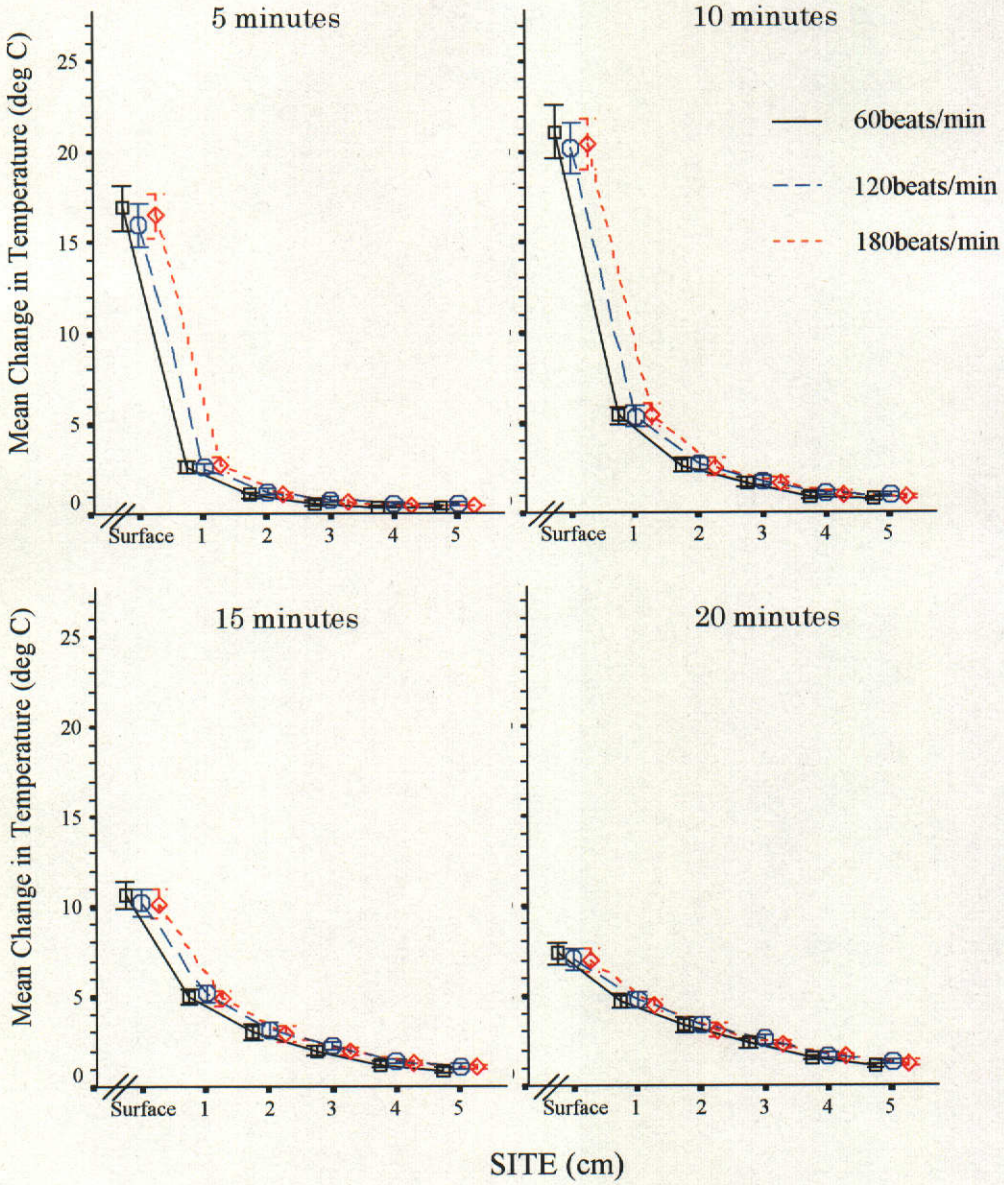

Figure 5.6: Mean (SE) change in temperature for three-way interactions among speed (slow – 60beats/min, moderate – 120beats/min, fast – 180beats/min), site (surface, 1, 2, 3, 4, 5 cm) and duration (5, 10, 15, 20 mins).

(2) Speed, Site and Duration

The results demonstrated that there was no significant 3-way interaction among speed, site and duration on the change in temperature ($F_{30, 270}$=1.318, p=0.13; Figure 5.6, Table 5.1). Regardless of the speed, the change in temperature was greater as the distance from the surface decreased, and as the duration of exposure increased, and conversely as the duration of post-exposure decreased.

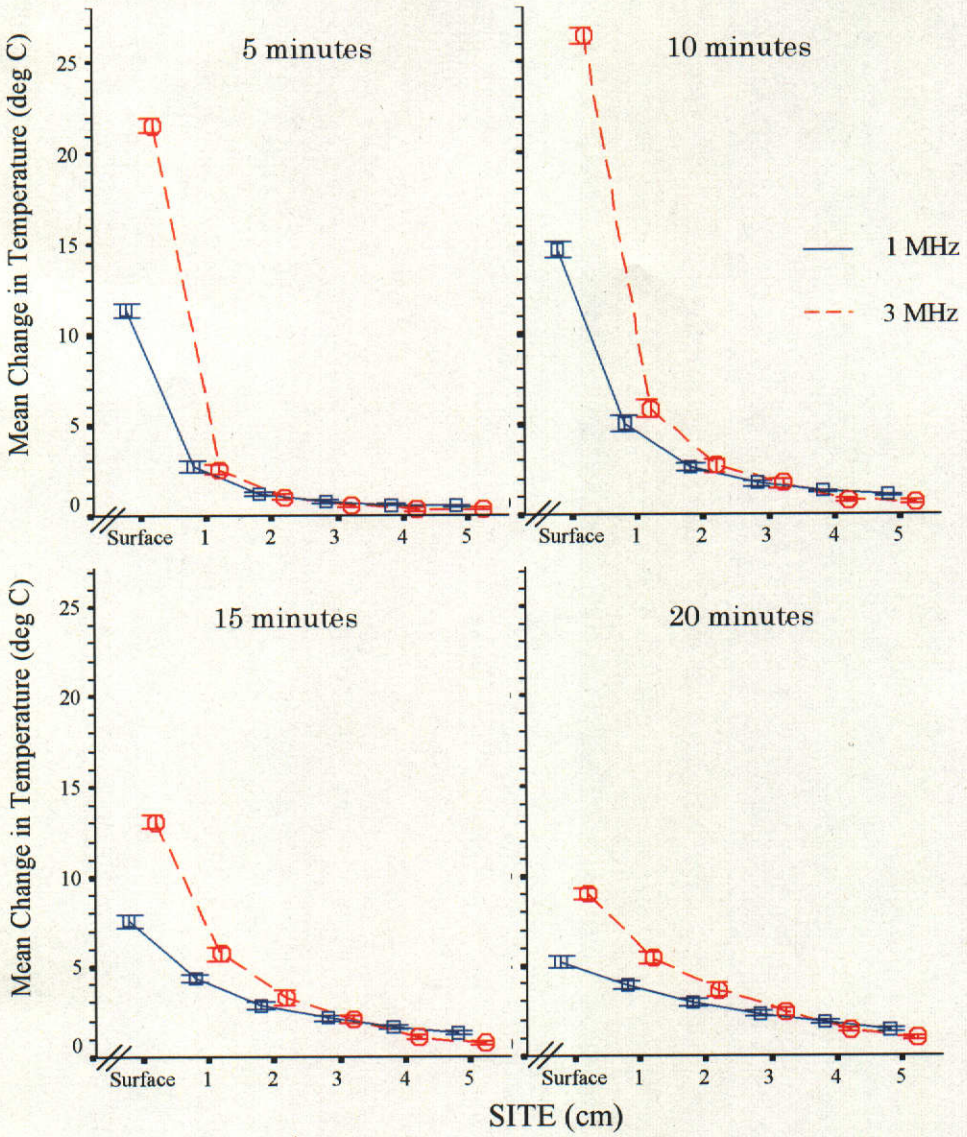

Figure 5.7: Mean (SE) change in temperature for three-way interactions among frequency (1, 3 MHz), site (surface, 1, 2, 3, 4, 5 cm) and duration (5, 10, 15, 20 mins).

(3) Frequency, Site and Duration

The results demonstrated that there was a significant 3-way interaction among frequency, site and duration on the change in temperature ($F_{15, 135}$=69.740, $p<0.01$; Figure 5.7, Table 5.1). The 3 MHz transducer produced greater increase in temperature than the 1 MHz transducer at the skin surface during the exposure phase (5 and 10 minutes), and at the skin surface and 1 cm below during the post-exposure phase (15 and 20 minutes).

A post-hoc analysis (repeated contrasts, within subjects, Table 5.1) demonstrated that there were significant interactions:

a. at 1 and 3 MHz and at 5 and 10 minutes

(1) between 1 and 2 cm ($F_{1,9}$=9.374, p=0.01).

This contrast showed that the increase in temperature from 5 to 10 minutes occurred more rapidly at 1 cm than at 2 cm, and this increase was greater for the 3 MHz than the 1 MHz transducer.

b. at 1 and 3 MHz and at 10 and 15 minutes

(1) between surface and 1 cm ($F_{1,9}$=236.742, p<0.01).

(2) between 2 and 3 cm ($F_{1,9}$=7.103, p=0.03).

These contrasts showed that the decrease in temperature from 10 to 15 minutes occurred more rapidly at the surface than at 1 cm, and more rapidly for the 3 MHz than for the 1 MHz transducer. This pattern was similar between 2 and 3 cm, although the magnitude of the change was less apparent.

c. at 1 and 3 MHz and at 15 and 20 minutes

(1) between surface and 1 cm ($F_{1,9}$=48.555, p<0.01)

(2) between 3 and 4 cm ($F_{1,9}$=5.435, p=0.05)

These contrasts showed that the decrease in temperature from 15 to 20 minutes occurred more rapidly at the surface than at 1 cm, and more rapidly for the 3 MHz than the 1 MHz transducer. This pattern was similar between 3 and 4 cm.

All other interactions were not significant (Table 5.1).

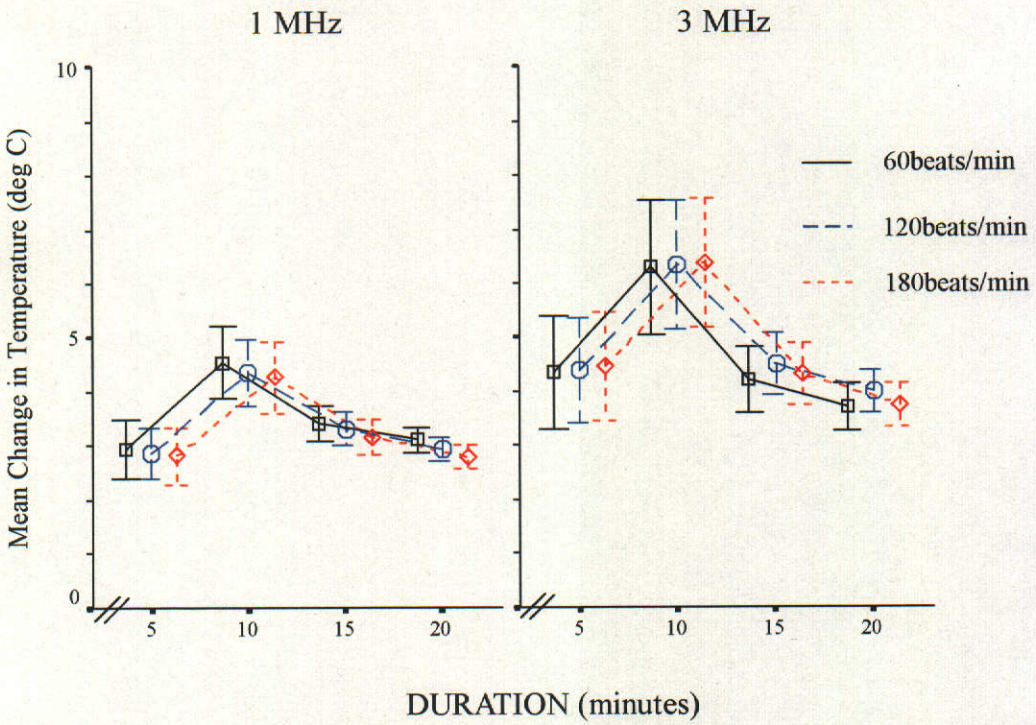

Figure 5.8: Mean (SE) change in temperature for three-way interactions among frequency (1, 3 MHz), speed (slow – 60beats/min, moderate – 120beats/min, fast – 180beats/min), and duration (5, 10, 15, 20 mins).

(4) Frequency, Speed and Duration

The results demonstrated that there was no significant 3-way interaction among frequency, speed and duration for the change in temperature ($F_{6, 54}$=0.803, p=0.57; Figure 5.8, Table 5.1). Regardless of the movement speed of the transducer, the rate of change in temperature from 5 to 10 minutes, 10 to 15 minutes, and 15 to 20 minutes, were similar although the magnitude of change in temperatures for the 3 MHz was greater the 1 MHz transducer.

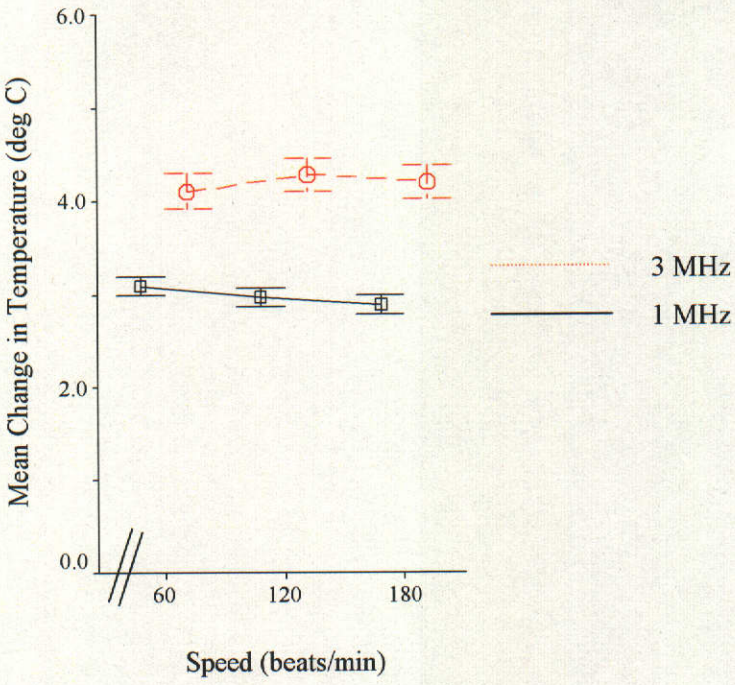

Figure 5.9: Mean (SE) change in temperature for two-way interactions between speed (slow – 60beats/min, moderate – 120beats/min and fast – 180beats/min) and frequency (1 & 3 MHz).

d. Results of analysis for two-way interactions

(1) Speed and Frequency

The results demonstrated that there was no significant 2-way interaction between speed and frequency on the change in temperature ($F_{2, 18}$=1.081, p=0.38; Figure 5.9, Table 5.2). The change in temperature was similar for all three movement speeds of the transducer, with the 3 MHz producing greater heating compared with the 1 MHz transducer at all three movement speeds.

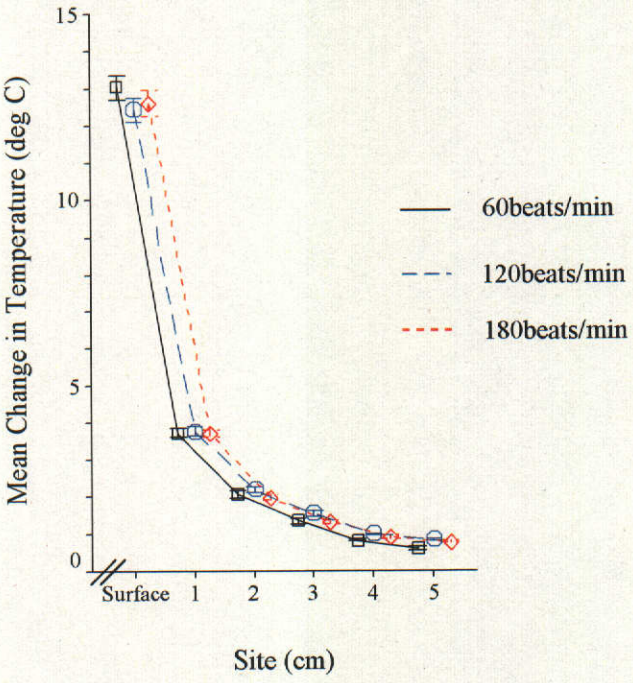

Figure 5.10: Mean (SE) change in temperature for two-way interactions between speed (slow – 60beats/min, moderate – 120beats/min and fast – 180beats/min) and site (surface, 1, 2, 3, 4, 5 cm).

(2) Speed and Site

The results demonstrated that there was no significant 2-way interaction between speed and site on the change in temperature ($F_{10, 90}$=1.641, p=0.11; Figure 5.10, Table 5.2). Regardless of the depth of the target tissue, the change in temperature was similar for all three movement speeds of the transducer.

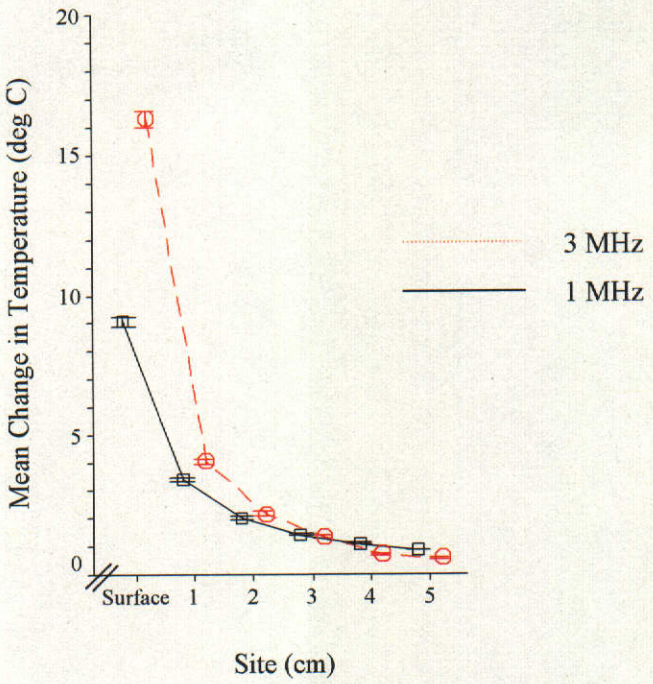

Figure 5.11: Mean (SE) change in temperature for two-way interactions between frequency (1 & 3 MHz) and site (surface, 1, 2, 3, 4, 5 cm).

(3) Frequency and Site

The results demonstrated that there was a significant 2-way interaction between frequency and site on the change in temperature ($F_{2.298, 20.686}$=104.123, $p<0.01$; Figure 5.11, Table 5.2). A post-hoc analysis (repeated contrasts, within subjects, Table 5.2) demonstrated that there were significant interactions at 1 and 3 MHz:

a. between surface and 1 cm ($F_{1, 9}$=205.376, $p<0.01$)

b. between 1 and 2 cm ($F_{1, 9}$=10.134, $p=0.01$).

These contrasts showed that the change in temperature was greater for the 3 MHz than the 1 MHz transducer, and that this was mainly confined to the superficial tissues between the surface and 1 cm, and between 1 and 2 cm. All other interactions were not significant (Table 5.2).

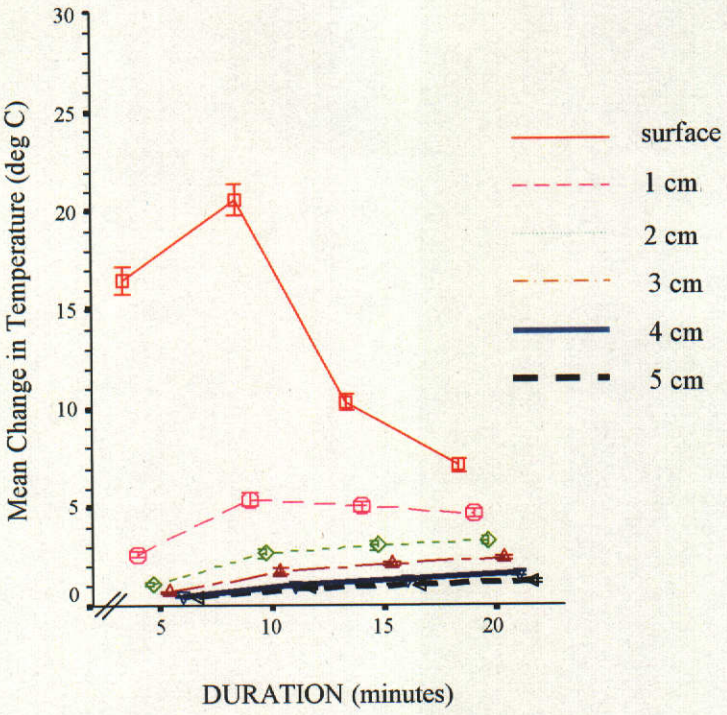

Figure 5.12: Mean (SE) change in temperature for two-way interactions between site (surface, 1, 2, 3, 4, 5 cm) and duration (5, 10, 15 and 20 mins).

(4) Site and Duration

The results demonstrated that there was a significant 2-way interaction between site and duration for the change in temperature ($F_{15, 135}$=612.151, $p<0.01$; Figure 5.12, Table 5.2). During the exposure phase (5 and 10 minutes), there was a progressive increase in temperature at all sites with increase in exposure time. During the post-exposure phase, there was a progressive decrease in temperature at the skin surface and 1 cm below the skin surface, and a progressive increase in temperature at 2, 3, 4 and 5 cm below the skin surface, with increase in post-exposure time. A post-hoc analysis (repeated contrasts, within subjects, Table 5.2) demonstrated that there were significant interactions:

a. at 5 and 10 minutes

(1) between surface and 1 cm ($F_{1, 9}$=36.073, $p<0.01$)

(2) between 1 and 2 cm ($F_{1,9}$=17.502, p<0.01)

(3) between 2 and 3 cm ($F_{1,9}$=23.234, p<0.01)

(4) between 3 and 4 cm ($F_{1,9}$=6.883, p=0.03)

(5) between 4 and 5 cm ($F_{1,9}$=9.862, p=0.01)

The above five contrasts indicated that the more superficial the site, the steeper the slope (or the faster the increase in temperature) between 5 and 10 minutes.

b. at 10 and 15 minutes

(1) between surface and 1 cm ($F_{1,9}$=999.900, p<0.01)

(2) between 1 and 2 cm ($F_{1,9}$=10.775, p=0.01)

(3) between 4 and 5 cm ($F_{1,9}$=6.111, p=0.04)

These contrasts showed that between 10 and 15 minutes, cooling at the surface was much quicker than cooling at any deeper site.

c. at 15 and 20 minutes

(1) between surface and 1 cm ($F_{1,9}$=1002.284, p<0.01)

(2) between 1 and 2 cm ($F_{1,9}$=43.362, p<0.01)

(3) between 2 and 3 cm ($F_{1,9}$=4.916, p=0.05)

(4) between 4 and 5 cm ($F_{1,9}$=13.553, p=0.01)

These contrasts showed that between 15 and 20 minutes, cooling at the surface was much quicker than cooling at any deeper site.

All other interactions were not significant (Table 5.2).

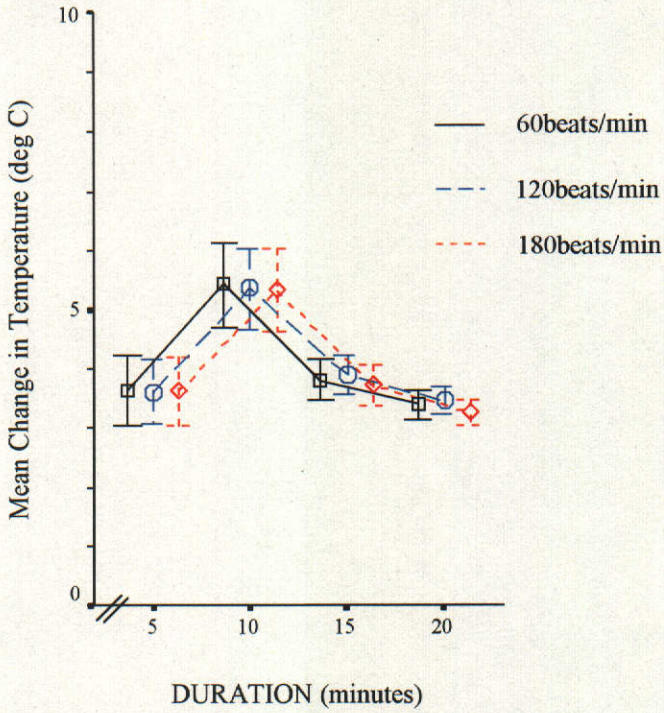

Figure 5.13: Mean (SE) change in temperature for two-way interactions between speed (slow – 60beats/min, moderate – 120beats/min and fast – 180beats/min) and duration (5, 10, 15 and 20 mins).

(5) Speed and Duration

The results demonstrated that there was no significant 2-way interaction between frequency and speed for the change in temperature ($F_{3.687,\ 33.179}$=1.803, p=0.16; Figure 5.13, Table 5.2). The increase in temperatures from 5 to 10 minutes, and the decrease in temperatures from 10 to 15 to 20 minutes were similar for all three movement speeds.

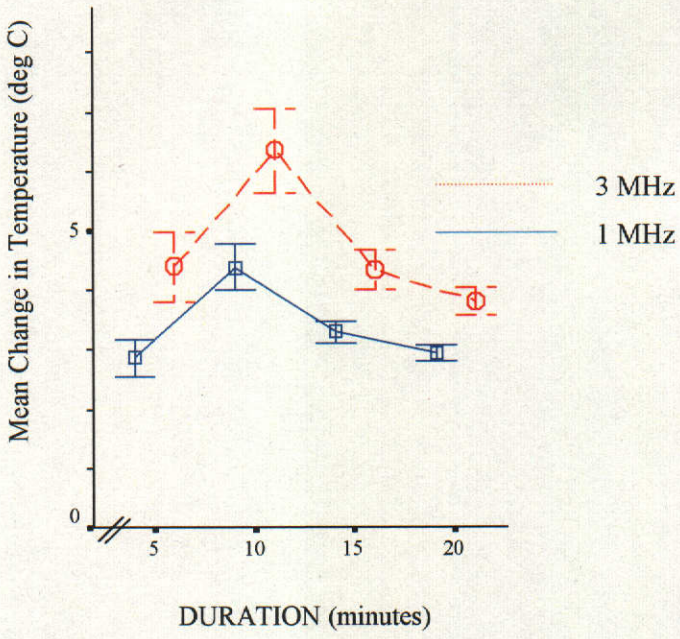

Figure 5.14: Mean (SE) change in temperature for two-way interactions between frequency (1, 3 MHz) and duration (5, 10, 15 and 20 mins).

(6) Frequency and Duration

The results demonstrated that there was a significant 2-way interaction between frequency and duration on the change in temperature ($F_{1.651, 14.859}$=19.198, $p<0.01$; Fig. 5.14, Table 5.2). The 3 MHz transducer produced greater increase in temperature compared with the 1 MHz transducer for both the exposure and post-exposure phases. A post-hoc analysis (repeated contrasts, within subjects, Table 5.2) demonstrated that there were significant interactions:

a. at 1 and 3 MHz

(1) between 10 and 15 minutes ($F_{1, 9}$=73.822, $p<0.01$)

(2) between 15 and 20 minutes ($F_{1, 9}$=6.881, $p=0.03$)

These contrasts showed that for the post-exposure phase (between 10 and 15, and between 15 and 20 minutes), cooling occurred more quickly for 3 MHz than for the 1 MHz transducer.

All other interactions were not significant (Table 5.2).

Table 5.2: Repeated Measures ANOVA (General Linear Model, within subjects effects), and Contrasts (within subjects, repeated) for the two-way and one-way interaction effects on main factors (Frequency: 1, 3 MHz; Speed: 60, 120, 180 bpm; Duration: 5, 10, 15, 20 mins; Site: 0 or surface, 1, 2, 3, 4, 5 cm).

Factor				df	F value	p value	Eta	Power
Freq	Speed			2	1.081	0.38	0.107	0.210
	Speed	Site		10	1.641	0.11	0.154	0.755
Freq		**Site**		2.298	104.123	<0.01	0.920	1.000
1 vs 3		5 vs 4		1	1.138	0.31	0.112	0.160
		4 vs 3		1	1.398	0.27	0.134	0.185
		3 vs 2		1	1.174	0.31	0.115	0.163
		2 vs 1		1	10.134	0.01	0.530	0.808
		1 vs 0		1	205.376	<0.01	0.958	1.000
Duration		**Site**		15	612.151	<0.01	0.986	1.000
5 vs 10		**5 vs 4**		1	9.862	0.01	0.523	0.798
		4 vs 3		1	6.883	0.03	0.433	0.647
		3 vs 2		1	23.234	<0.01	0.721	0.989
		2 vs 1		1	17.502	<0.01	0.660	0.960
		1 vs 0		1	36.073	<0.01	0.800	1.000
10 vs 15		**5 vs 4**		1	6.111	0.04	0.404	0.597
		4 vs 3		1	0.880	0.37	0.089	0.134
		3 vs 2		1	0.065	0.81	0.007	0.056
		2 vs 1		1	10.775	0.01	0.545	0.831
		1 vs 0		1	999.900	<0.01	0.991	1.000
15 vs 20		**5 vs 4**		1	13.553	0.01	0.601	0.905
		4 vs 3		1	0.236	0.64	0.026	0.072
		3 vs 2		1	4.916	0.05	0.353	0.508
		2 vs 1		1	43.362	<0.01	0.828	1.000
		1 vs 0		1	1002.284	<0.01	0.991	1.000
Duration	Speed			3.687	1.803	0.16	0.167	0.470
Duration	**Freq**			1.651	19.198	<0.01	0.681	0.998
5 vs 10	1 vs 3			1	3.132	0.111	0.258	0.353
10 vs 15	**1 vs 3**			1	73.822	<0.01	0.891	1.000
15 vs 20	**1 vs 3**			1	6.881	0.03	0.433	0.647
	Speed			2	0.529	0.60	0.055	0.124
Freq				1	11.193	0.01	0.554	0.845
		Site		2.400	367.922	<0.01	0.976	1.000
		5 vs 4		1	25.971	<0.01	0.743	0.994
		4 vs 3		1	9.557	0.01	0.515	0.785
		3 vs 2		1	21.656	<0.01	0.706	0.984
		2 vs 1		1	35.856	<0.01	0.799	1.000
		1 vs 0		1	1199.380	<0.01	0.993	1.000
Duration				1.395	153.042	<0.01	0.944	1.000
5 vs 10				1	145.613	<0.01	0.942	1.000
10 vs 15				1	1167.699	<0.01	0.992	1.000
15 vs 20				1	139.440	<0.01	0.939	1.000

Freq = Frequency; df = degrees of freedom; Eta = Estimated effect size; bpm = beats per minute. (Note: Bold fonts indicate significant effects or interactions)

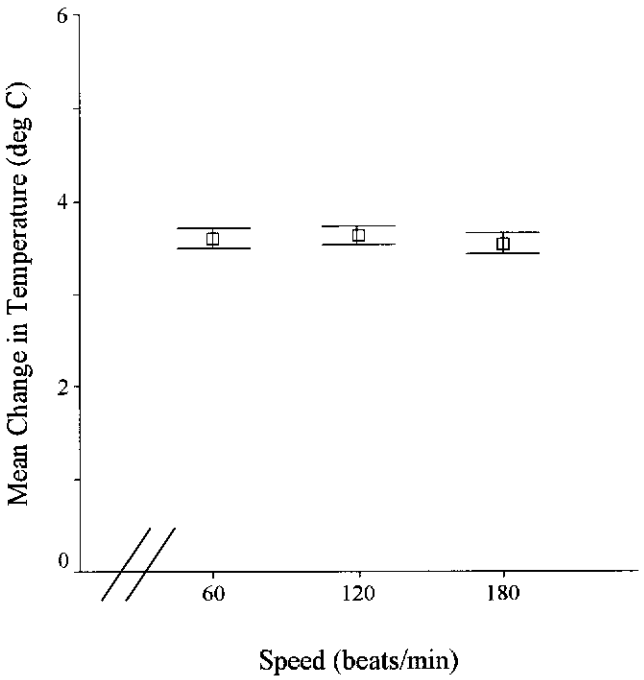

Figure 5.15: Mean (SE) change in temperature for main effects of speed (slow – 60beats/min, moderate – 120beats/min, and fast – 180beats/min).

e. Results of analysis for main factors

(1) Speed

The results demonstrated that varying the speed had no significant effect on the change in temperature ($F_{2, 18}$=0.529, p=0.60; Figure 5.15, Table 5.2). The change in temperature for all three movement speeds of the transducer was similar.

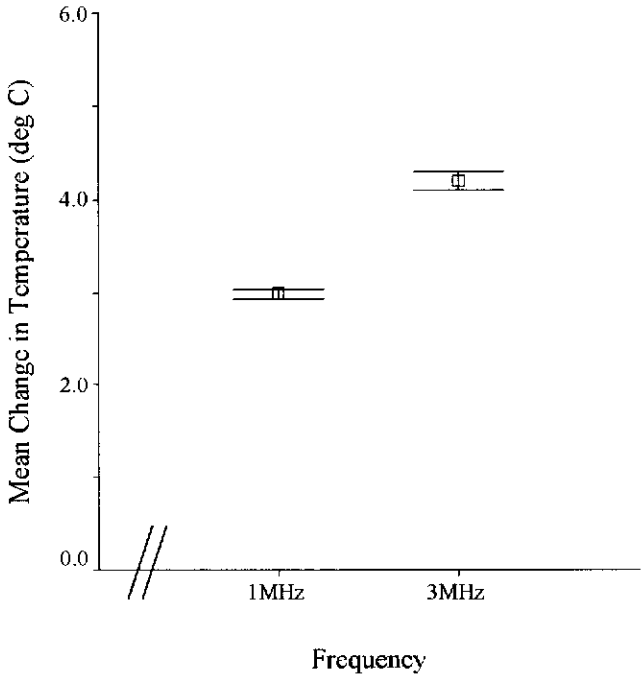

Figure 5.16: Mean (SE) change in temperature for main effects of frequency (1MHz, 3MHz).

(2) Frequency

The results demonstrated that varying the frequency (1 MHz versus 3 MHz) had a significant effect on the change in temperature ($F_{1,9}$=11.193, p=0.01; Figure 5.16, Table 5.2). The 3 MHz transducer produced greater increase in temperature compared with the 1 MHz transducer.

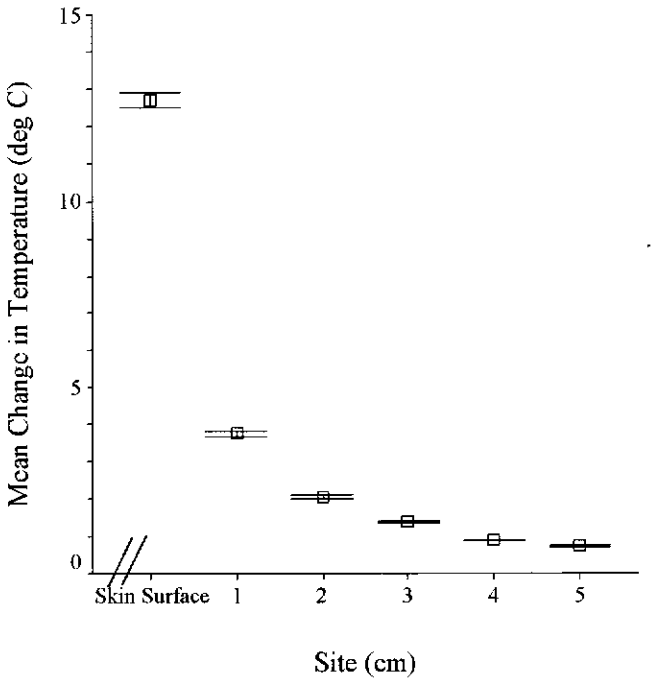

Figure 5.17: Mean (SE) change in temperature for main effects of site (skin surface, and 1, 2, 3, 4, and 5 cm below surface).

(3) Site

The results demonstrated that varying the depth of the target tissue had a significant effect on the change in temperature ($F_{2.400, 21.602}$=367.922, $p<0.01$; Figure 5.17, Table 5.2). As the distance from the skin surface increased, the change in temperature decreased. A post-hoc analysis (repeated contrasts, within subjects, Table 5.2) demonstrated that there were significant differences:

a. between surface and 1 cm ($F_{1, 9}$=1199.380, $p<0.01$)

b. between 1 and 2 cm ($F_{1, 9}$=35.856, $p<0.01$)

d. between 2 and 3 cm ($F_{1, 9}$=21.656, $p<0.01$)

e. between 3 and 4 cm ($F_{1, 9}$=9.557, $p=0.01$)

f. between 4 and 5 cm ($F_{1, 9}$=25.971, $p<0.01$)

The above contrasts showed that the change in temperature at each adjacent tissue site was significantly different.

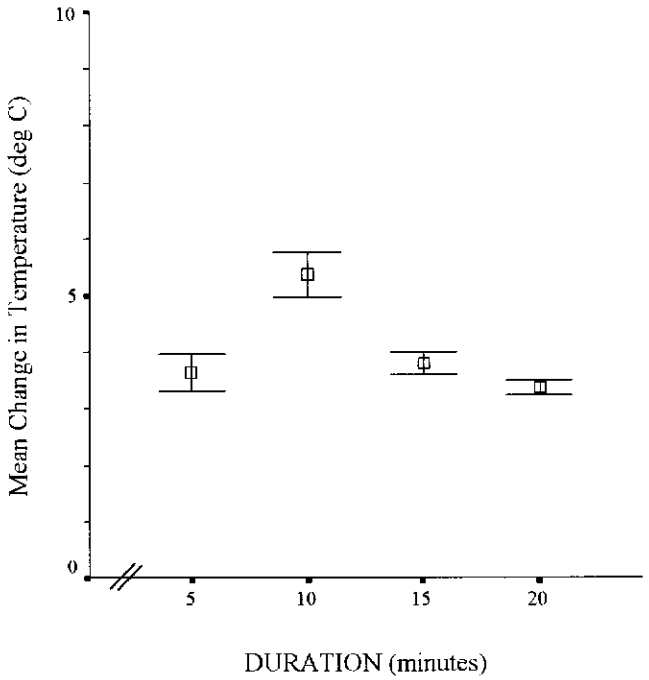

Figure 5.18: Mean (SE) change in temperature for main effects of duration of exposure (5, 10 mins) and post-exposure (15, 20 mins).

(4) Duration

The results demonstrated that varying the duration of exposure (5 and 10 minutes) and post-exposure (15 and 20 minutes) had a significant effect on the change in temperature ($F_{1.395,\ 12.551}$=153.042, $p<0.01$; Figure 5.18, Table 5.2). As the duration of exposure increased, there was a progressive increase in temperature. Conversely, as the duration of post-exposure increased, there was a progressive decrease in temperature. A post-hoc analysis (repeated contrasts, within subjects, Table 5.2) demonstrated that there were significant differences:

a. between 5 and 10 minutes ($F_{1,\ 9}$=145.613, $p<0.01$)

b. between 10 and 15 minutes ($F_{1,\ 9}$=1167.699, $p<0.01$)

c. between 15 and 20 minutes ($F_{1,\ 9}$=139.440, $p<0.01$)

f. Other results

The means and standard deviations for change in temperature at the skin surface, and 1, 2, 3, 4 and 5 cm below the skin are included in Tables A4-1, A4-2, A4-3, A4-4, A4-5 and A4-6 respectively (see Appendix 4). The thermographic isothermic scans of specimens after 10 minutes exposure and 5 minutes post-exposure to a 3 MHz ultrasound are also included (Figure A4-1, Appendix 4).

5.5 DISCUSSION

a. General

Figures 5.2 and 5.3 illustrate the change in temperatures during the exposure phase (1 to 10 minutes) and the post-exposure phase (11 to 20 minutes) for 1 and 3 MHz frequencies respectively.

The results of this study for 1 MHz frequency (Figure 5.2) were very similar to the reliability study at the same frequency (Chapter 4) in terms of the increase in temperatures, as well as the pattern of heating and cooling obtained at the various tissue sites (see Figure 5.2, p126; and Figure 4.1, p107). For the exposure phase, the change in temperature increased as the duration of exposure increased, up to the final 10^{th} minute. This magnitude of increase was also related to the depth of the target tissue, with peak temperatures decreasing as the depth increased (Figures 5.2 and 5.3). For the post-exposure phase (11 to 20 minutes), the pattern for the 1 MHz frequency was exactly the same as that found in the reliability study (Chapter 4, pp 112-3). In summary, there were two discernible patterns for the superficial tissues (surface, 1 and 2 cm below surface) and for the deep tissues (3, 4 and 5 cm below surface). At the skin surface and at 1 cm below surface, there was a progressive decrease of the temperatures. At 2 cm below surface, the temperature "leveled off" at around the peak temperature, and remained relatively constant throughout the

entire post-exposure phase. For the deep tissues at the 3, 4 and 5 cm below surface, there was a gradual increase in temperature (less than 1°C) over the entire 10 minutes post-exposure phase.

Compared with the 1 MHz frequency, the heating pattern for the 3 MHz frequency for this study demonstrated similar features. The main difference for the 3 MHz frequency, however, was that the "leveling off" of the temperature change in the post-exposure phase occurred more superficially at 1 cm rather than 2 cm. Furthermore, while the 1 and 3 MHz frequencies demonstrated a similar pattern of heating and cooling, the increase in temperatures at each tissue site was markedly different. This difference was most pronounced at the skin surface, with the 3 MHz producing greater increase in temperatures than the 1 MHz frequency: peak temperature for the 3 MHz was almost twice as great as the 1 MHz frequency. At 1 and 2 cm below the skin surface, the increase in temperature for the 3 MHz was slightly greater than the 1 MHz, with the differences in peak temperatures becoming less the further the distance from the skin surface. At 3 cm below the skin surface, there was almost no difference in the temperature increase between the two frequencies. However, at 4 and 5 cm below the skin surface, the increase in temperature was slightly greater for the 1 MHz compared with the 3 MHz frequency.

The "leveling off" at 1 cm for the 3 MHz compared with 2 cm for the 1 MHz, and the subsequent lesser increase in temperature at 2, 3, 4 and 5 cm below the skin surface for the 3 MHz frequency, could be an indication of the difference in penetration depth between the two frequencies. Therefore, at 1.0 Watt/cm^2 intensity, it appears that the 1 MHz frequency was able to penetrate deeper than the 3 MHz frequency, with the former producing greater increase in temperatures at the deeper tissues. This can be attributed to the lower heating at the skin surface from the 1 MHz frequency, thereby conserving sufficient energy that is transmitted and

absorbed at deeper sites. In comparison, the 3 MHz frequency appeared to favor greater heating at the skin surface, with less energy available to transmit to the deeper tissues. Whether this relationship holds true for the other intensities is unknown and will be investigated in the main study (Chapter 8).

b. Speed and its interactions

The main aim of this study was to examine the relationship between speed and frequency, site of the target tissue and duration of exposure / post-exposure. The results of this study clearly demonstrated that there was no significant four-way, three-way and two-way interactions or main effects of speed on the temperature increase. Varying the movement speed of the transducer from slow, (60 beats/min) to moderate (120 beats/min) to fast (180 beats/min), had no effect on the change in temperature, either at the surface or within the tissues up to a depth of 5 cm, for both 1 and 3 MHz, during both exposure and post-exposure phases. Part of the reason for this result could be because the transducer was always moved within a specified area determined by the thermoplastic guide. The size of the exposed area was twice the size of the transducer or effective radiating area (2X ERA). Therefore, the ultrasonic energy that was delivered to the same volume of tissues below this thermoplastic guide was always constant and hence, the heating produced within the tissues was also constant, regardless of the speed of movement of the transducer. This is also demonstrated quite clearly from the thermographic scans for 3 MHz (Figure A4-1, Appendix 4).

This main finding has implications for subsequent studies as well as clinical practice. As long as the ultrasonic transducer is moved at speeds greater than 60 beats/min and less than 180 beats/min, the increase in temperatures at the skin surface and up to a depth of 5 cm below can be expected to be generally unaffected. However, the investigators found that the most comfortable speed for the operator of

the ultrasound to move the transducer was at 120 beats/minute (medium speed) and this is the recommended speed for moving the transducer in future studies.

c. Other results

The results of this study supported the expectation that the heating pattern would be different for 1 and 3 MHz, but this appeared to be only at the skin surface. The subcutaneous temperatures from 1 cm to 5 cm were not significantly different between the two frequencies (Table 5.2, Figures 5.4 and 5.6). However, if the assumption is made that an increase in temperature of at least 1°C is clinically important, this study demonstrated that 1 MHz ultrasound for 10 minutes at 1.0 Watt/cm^2 was capable of increasing tissue temperatures by 1°C up to a tissue depth of 5 cm. On the other hand, 3 MHz ultrasound for 10 minutes at 1.0 Watt/cm^2 was capable of increasing tissue temperatures by 1°C only to a tissue depth of 3cm (see Appendix 4). Hence, this study demonstrated that 1 MHz ultrasound was capable of heating target tissues at a depth greater than 3 MHz ultrasound. However, this was only at an intensity of 1.0 Watt/cm^2. Whether this relationship holds true for the other intensities remains to be investigated.

5.6 CONCLUSION

The main conclusion from this study was that varying the movement speed of the transducer, greater than 60 beats/min and less than 180 beats/min, did not have an effect on the temperature increase within the tissues exposed to ultrasound, up to a depth of 5 cm, regardless of both the frequency of the transducer and duration of exposure / post-exposure. Therefore, all the null hypotheses (1 and 2) for this study were accepted. However, the investigator found that the most comfortable speed for the operator of the ultrasound to move the transducer was at 120 beats/minute (medium speed) and this is the recommended speed for moving the transducer in future studies, as well as in clinical practice.

CHAPTER SIX

THE EFFECT OF VARYING THE SIZE OF THE TREATMENT AREA (SMALL, MEDIUM AND LARGE) ON TEMPERATURE INCREASES IN POST-MORTEM PIG TISSUES EXPOSED TO THERAPEUTIC ULTRASOUND

6.1 INTRODUCTION

The size of treatment area for a therapeutic ultrasound application usually varies with the application, pathology and anatomical site. The size of treatment area is usually measured in multiples of the surface area of the transducer. If more accurate measurements are required, the size of the effective radiating area (ERA) is used instead of the surface area of the transducer. On average, most treatment applications involve at least twice the size of the ERA (2X ERA), but some applications (such as over the shoulder or back) can cover as much as four to eight times the size of ERA (4X to 8X ERA).

Chan et al (1998) investigated the effect of varying the size of the treatment area on temperature changes in the human patellar tendon. The patellar tendons of 8 healthy subjects were exposed to ultrasound (3 MHz frequency, 1 Watt/cm^2 intensity, 4 minutes exposure time) for two different treatment sizes corresponding to twice (2X ERA) and four times the ERA (4X ERA) of the transducer. Increases in tissue temperature were recorded at 1 cm depth from the skin surface using a

thermistor probe. Their results showed that the mean increase in temperature at the end of four minutes were significantly different for the two treatment sizes (8.3°C for the 2X ERA, and 5.0°C for the 4X ERA). Chan et al (1998) concluded that the smaller (2X ERA) treatment size was more effective and provided higher and longer heating than the larger (4X ERA) treatment size.

Chan et al (1998) has reported the only study that investigated the effect of varying the size of treatment area on amount of heating in the subcutaneous tissues. However, only one frequency (3 MHz) was investigated, at one tissue depth (1cm) and comparison was made between only two treatment area sizes (2X and 4X ERA). Nevertheless, the results of the Chan et al's study (1998) highlighted the importance of standardizing the treatment size in order to maintain consistency in treatment applications.

While the main aim of this thesis was to examine the relationship between the frequency, the output intensity and the duration of exposure to therapeutic ultrasound on increase in temperature at various distances from the skin surface, it was unclear whether varying the size of the treatment area could be a confounding factor. While Chan et al's study (1998) was able to clarify the fact that smaller treatment areas (2X ERA) were more effective than larger treatment areas (4X ERA) in increasing tissue temperatures at 1 cm depth for a 3 MHz ultrasound machine, it was unclear if this relationship was also true for a 1 MHz frequency ultrasound machine, and at tissue depths greater than 1 cm. This information was necessary in order to minimise any confounding factors that could influence the main study.

The purpose of this next study was to determine the effect of varying the size of the treatment area (small or 2X ERA, medium or 3X ERA, and large or 4X ERA) on temperature increases at various distances from the skin surface in post-mortem pig tissues exposed to a 1 MHz frequency ultrasound machine.

6.2 NULL HYPOTHESES

The null hypotheses for this study were:

1. Varying the size of treatment area (small or 2X ERA, medium or 3X ERA, and large or 4X ERA) does not affect temperature increases in post-mortem pig tissues exposed to a 1MHz frequency therapeutic ultrasound at:
   a. the skin surface,
   b. 1 cm below the skin surface,
   c. 2 cm below the skin surface,
   d. 3 cm below the skin surface
   e. 4 cm below the skin surface
   f. 5 cm below the skin surface.

6.3 METHODS

a. Specimens

Fifteen specimens, obtained from post- mortem adult domestic pigs, were processed as previously described (Chapter 3, section 3.4, pp92-94). This experiment entailed testing at 1 frequency and 3 sizes of treatment area. Each specimen was used three times, once at each of the three sizes of treatment area being tested in this study. Specimens were left for at least an hour at room temperature between applications to allow them to return to baseline temperature.

b. Procedure and Instrumentation

The instrumentation and general procedure have been described in Chapter 3 (sections 3.3 and 3.4, pp88-100). In summary, therapeutic ultrasound at 1 MHz

frequency was applied to the tissues at 1.0 Watt/cm^2 for 10 minutes. The movement speed of the transducer was standardized at 120 beats/minute (moderate speed) as recommended by the results of a previous study (see Chapter 5) and this was maintained with a metronome. The size of the treatment area varied from small (2X ERA) to medium (3X ERA) to large (4X ERA) and was maintained using a thermoplastic guide (see Figure 6.1), secured over the shaved skin of the designated treatment area. A direct in-contact moving soundhead technique using ultrasonic gel was applied.

The order of selection for the three sizes of treatment area was randomized. For the first size of treatment area selected, increases in tissue temperatures at six sites (skin surface, and 1, 2, 3, 4 and 5 cm below the skin surface) were monitored at baseline (prior to exposure) and subsequently at one-minute intervals during the 10-minutes exposure, as well as for another 10 minutes post-exposure. Surface skin temperature was recorded with the infrared spot thermometer (Minolta HT-11) while the subcutaneous temperatures (1, 2, 3, 4 and 5 cm below skin surface) were measured with the infrared thermography unit (Avio TVS 2000). The test-retest reliability of the infrared spot thermometer and the infrared video thermography unit had been previously established (see Chapter 4).

At the end of 20 minutes, the tissue specimen was left standing at room temperature to cool down for at least an hour. The experimental setup was left in situ. For measurements of the second and third sizes of treatment area, the experimental procedure was repeated using exactly the same parameters and procedure. In this manner, all experiments for all three sizes of treatment area were performed on one tissue specimen. The same investigator performed all the measurements. The general procedure is summarized in Figure 3.1 (Chapter 3, p86). The specific procedure for this study is summarized in Figure 6.2 (p155).

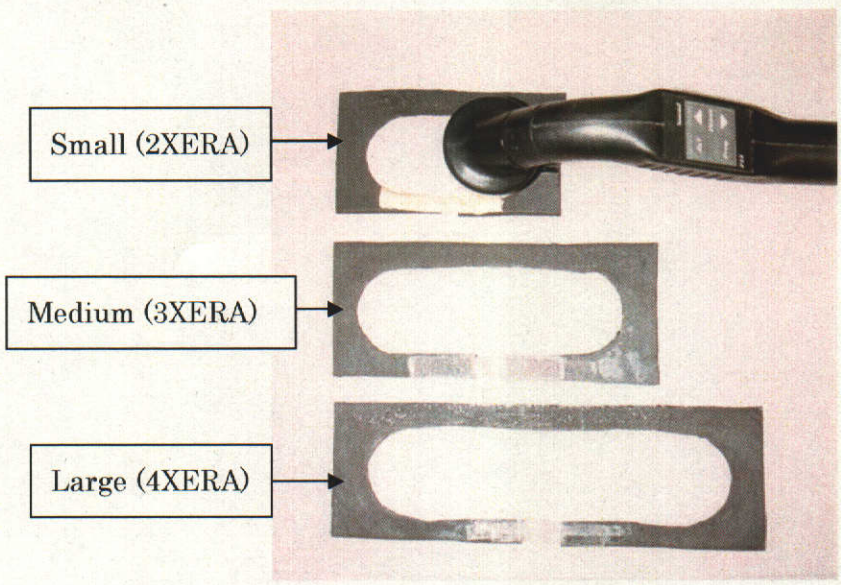

Figure 6.1: Thermoplastic guides corresponding to the various sizes of treatment area

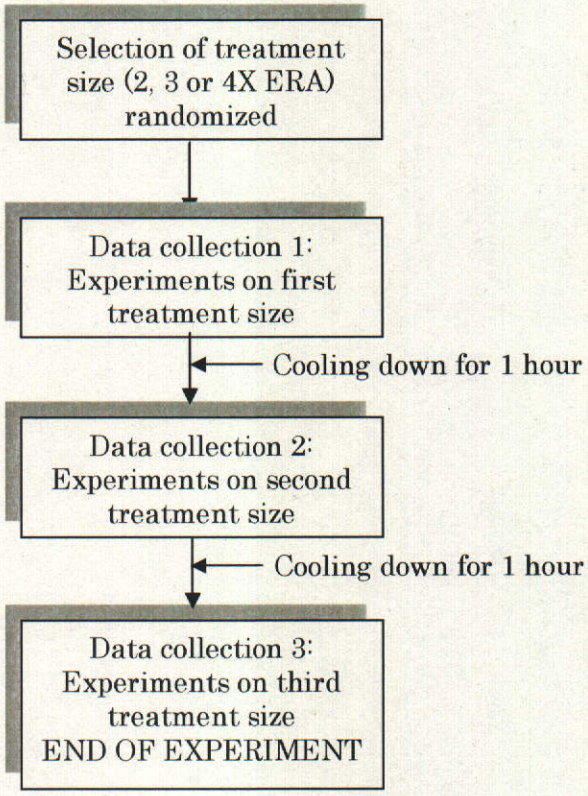

Figure 6.2: Flow chart of specific procedure.

At the end of the experiments, the specimens were disposed of according to the procedure described in Chapter 3 (section 3.4, paragraph j, p100).

c. Data Analysis

A general description for data analysis has been provided in Chapter 3 (section 3.5, p101).

Data from all fifteen specimens were analysed. Analysis of the data was performed using change in temperature (dependent variable), rather than actual temperature measured, at the 5^{th}, 10^{th}, 15^{th}, and 20^{th} minutes. The reliability at these four time periods was found to be representative of the exposure and the post-exposure phases (Chapter 4). However, data for the entire 20-minute sampling are provided in the tables of means (Appendix 5) and Figure 6.3.

A three (size – small, medium large) by six (sites – skin surface, and 1, 2, 3, 4, 5 cm below skin surface) by four (duration – 5, 10, 15, 20 minutes) repeated measures analysis of variance was used to assess the effect of varying the treatment size on the change in tissue temperatures (dependent variable) on the skin surface, as well as at five tissue depths. For those factors that were found to be significantly different, a repeated contrasts (within subjects) post-hoc analysis on factors that had greater than two levels was performed to identify differences among the levels.

In addition, the observed power, the estimates of effect size and descriptive statistics for means and standard deviations of the scores were also calculated. The assumptions of sphericity, equal variance and normality of the statistical procedures were checked.

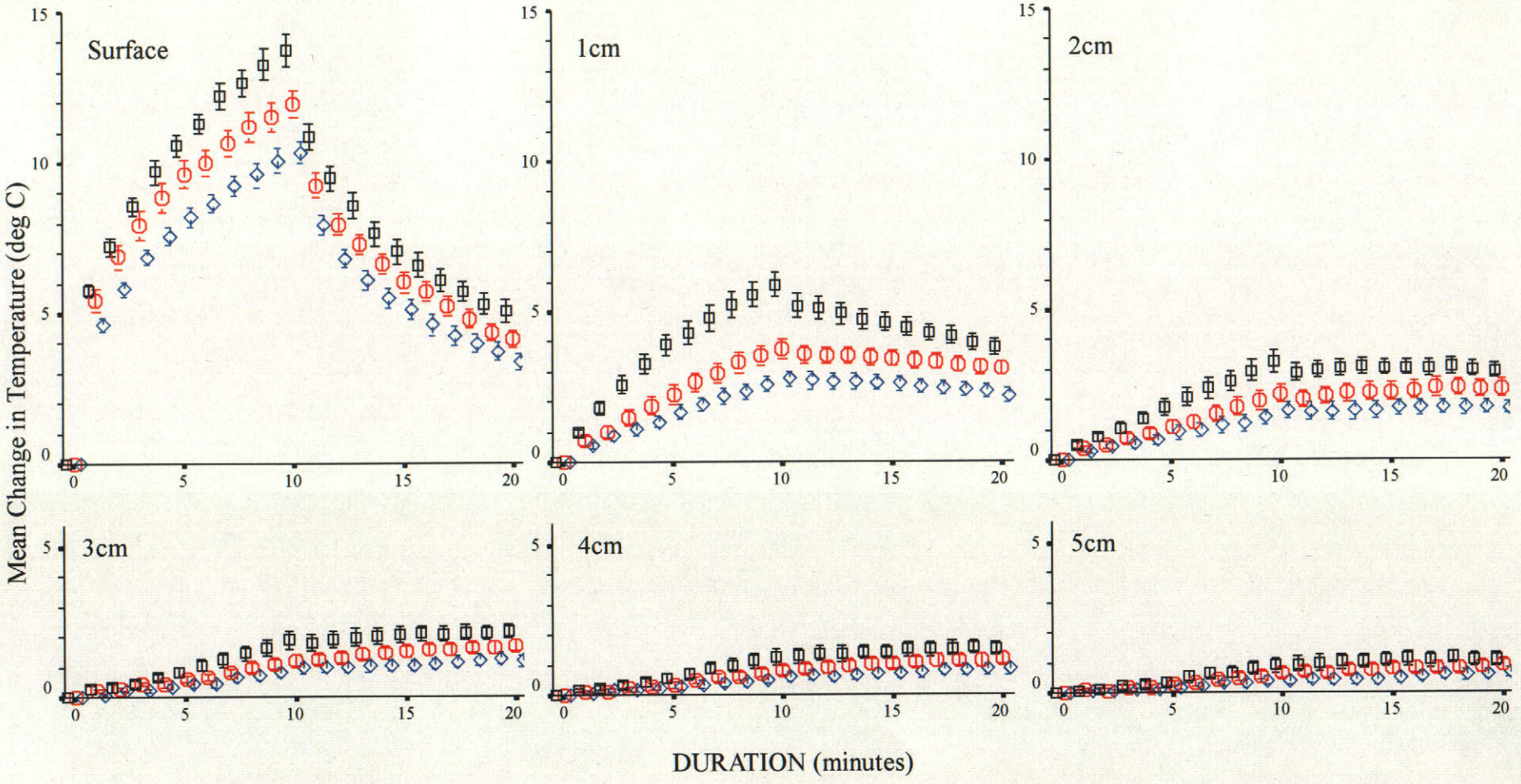

Figure 6.3: Mean change in temperatures for 10 minutes exposure and 10 minutes post-exposure to ultrasound at 1MHz, 1.0 Watts/cm^2 for small (2X ERA), medium (3X ERA) and large (4X ERA) treatment areas.

6.4 RESULTS

The main factor of interest in the analysis was size of treatment area and its interactions. A general description of the results will be presented first. Following this, the details of these results for size, site and duration will be presented for their three-way interactions, followed by their two-way interactions, and finally for their main effects.

a. General

The change in temperature over time for small, medium and large size of treatment area at the skin surface, and at 1, 2, 3, 4 and 5 cm below the skin is shown in Figure 6.3. The largest change in temperature occurred at the skin surface for all three sizes of treatment area. The peak increase in temperature at the skin surface was greatest for the small treatment area (13.75°C), and this decreased as the size of treatment area increased from small to medium (11.95°C) to large (10.35°C) (Figure 6.3, and Table A5-1 in Appendix 5). The differences in change in temperature between small, medium and large size of treatment area also decreased as the depth of target tissue increased (see Figure 6.3).

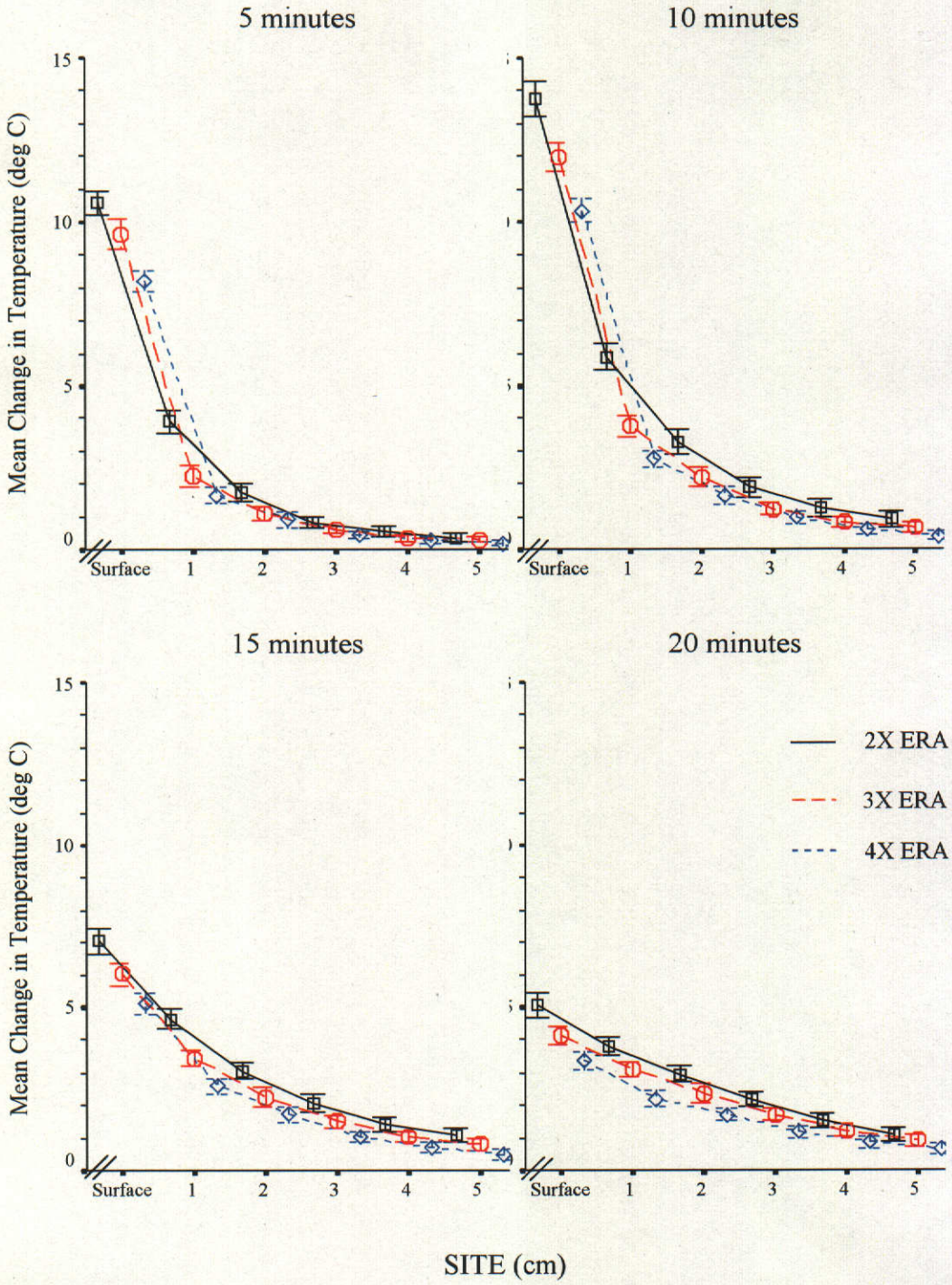

Figure 6.4: Mean (SE) change in temperature for three-way interactions among size of treatment area (2X ERA - small, 3X ERA - medium, and 4X ERA – large), site (surface, 1, 2, 3, 4, 5 cm), and duration (5, 10, 15 and 20 minutes).

b. Results of analysis for three-way interactions

(1) Size, Site and Duration

The results demonstrated that there was a significant 3-way interaction among size of treatment area, site of target tissue and duration of exposure / post-exposure on the change in temperature ($F_{30, 420}$=6.244, $p<0.01$; Figure 6.4, Table 6.1). During the exposure phase (5 and 10 minutes), as the duration increased, and as the distance from the surface and the size of treatment area decreased, there was greater increase in temperature. During the post-exposure phase (15 and 20 minutes), as the duration, the distance from the surface and the size of the treatment area decreased there was greater increase in temperature. A post-hoc analysis (repeated contrasts, within subjects, Table 6.1) demonstrated that there were significant interactions:

a. at 2X and 3X ERA and at 5 and 10 minutes

(1) between 3 and 4 cm ($F_{1, 14}$=19.070, $p<0.01$)

The contrast shows that as the duration of exposure increased (between 5 and 10 minutes), there was greater increase in temperature at 2X ERA compared with 3X ERA between 3 and 4 cm tissue depths.

b. at 2X and 3X ERA and at 10 and 15 minutes

(1) between 1 and 2 cm ($F_{1, 14}$=15.627, $p<0.01$)

(2) between 4 and 5 cm ($F_{1, 14}$=4.613, $p=0.05$)

The contrasts show that as the duration of post-exposure increased (between 10 and 15 minutes), there was greater decrease in temperature at 2X ERA compared with 3X ERA between 1 and 2 cm, and between 4 and 5 cm tissue depths.

c. at 3X and 4X ERA and at 10 and 15 minutes

(1) between surface and 1 cm ($F_{1, 14}$=5.447, p=0.04)

(2) between 2 and 3 cm ($F_{1, 14}$=6.370, p=0.02)

(3) between 3 and 4 cm ($F_{1, 14}$=4.849, p=0.05)

The contrasts show as the duration of post-exposure increased (between 10 and 15 minutes), there was greater decrease in temperature at 3X ERA compared with 4X ERA between the adjacent tissues from the surface up to a depth of 4 cm.

d. at 2X and 3X ERA and at 15 and 20 minutes

(1) between 1 and 2 cm ($F_{1, 14}$=6.664, p=0.02)

The contrast shows that as the duration of post-exposure increased (between 15 and 20 minutes), there was greater decrease in temperature at 2X ERA compared with 3X ERA between 1 and 2 cm tissue depths.

All other interactions were not significant (Table 6.1).

Table 6.1: Repeated Measures ANOVA (General Linear Model, within subjects effects), and Contrasts (within subjects, repeated) for the three-way interaction effects on main factors (Size: 2X, 3X, 4X ERA; Duration: 5, 10, 15, 20 mins; Site: 5, 4, 3, 4, 1, 0 cm).

Factor			df	F value	p value	Eta	Power
Duration	**Size**	**Site**	30	6.244	<0.01	0.308	1.000
5 vs 10	**2X vs 3X**	5 vs 4	1	0.244	0.63	0.017	0.075
		4 vs 3	1	19.070	<0.01	0.577	0.982
		3 vs 2	1	0.031	0.86	0.002	0.053
		2 vs 1	1	0.059	0.81	0.004	0.056
		1 vs 0	1	2.138	0.17	0.132	0.276
	3X vs 4X	5 vs 4	1	0.317	0.58	0.022	0.082
		4 vs 3	1	0.736	0.41	0.050	0.126
		3 vs 2	1	2.292	0.15	0.141	0.292
		2 vs 1	1	0.159	0.70	0.011	0.066
		1 vs 0	1	1.080	0.32	0.072	0.163
10 vs 15	**2X vs 3X**	**5 vs 4**	1	4.613	0.05	0.248	0.516
		4 vs 3	1	0.436	0.52	0.030	0.095
		3 vs 2	1	1.384	0.26	0.090	0.195
		2 vs 1	1	15.627	<0.01	0.527	0.956
		1 vs 0	1	0.095	0.76	0.007	0.060
	3X vs 4X	5 vs 4	1	0.935	0.35	0.063	0.147
		4 vs 3	1	4.849	0.05	0.257	0.536
		3 vs 2	1	6.370	0.02	0.313	0.651
		2 vs 1	1	2.311	0.15	0.142	0.294
		1 vs 0	1	5.447	0.04	0.280	0.584
15 vs 20	**2X vs 3X**	5 vs 4	1	1.098	0.31	0.073	0.165
		4 vs 3	1	0.478	0.50	0.033	0.099
		3 vs 2	1	2.510	0.14	0.152	0.315
		2 vs 1	1	6.664	0.02	0.322	0.671
		1 vs 0	1	2.214	0.16	0.137	0.284
	3X vs 4X	5 vs 4	1	0.405	0.54	0.028	0.091
		4 vs 3	1	0.272	0.61	0.019	0.078
		3 vs 2	1	0.732	0.41	0.050	0.126
		2 vs 1	1	0.522	0.48	0.036	0.103
		1 vs 0	1	1.316	0.27	0.086	0.188

df = degrees of freedom; Eta = Estimated effect size **(Note: Bold fonts indicate significant effects or interactions)**

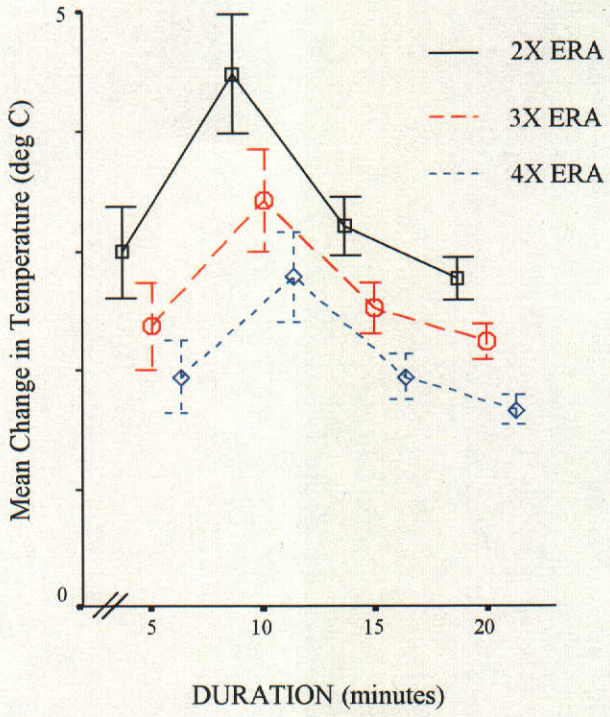

Figure 6.5: Mean (SE) change in temperature for two-way interactions between size of treatment area (2X ERA - small, 3X ERA - medium, and 4X ERA – large) and duration (5, 10, 15 and 20 minutes).

c. Results of analysis for two-way interactions

(1) Size and Duration

The results demonstrated that there was a significant 2-way interaction between size of treatment area and duration of exposure on the change in temperature ($F_{4.605, 64.475}$=12.855, $p<0.01$; Figure 6.5, Table 6.2). During the exposure phase (5 and 10 minutes), as the duration increased and size of the treatment area decreased, there was greater increase in temperature. During the post-exposure phase (15 and 20 minutes), as the duration and the size of the treatment area decreased there was greater decrease in temperature. A post-hoc analysis (repeated contrasts, within subjects, Table 6.2) demonstrated that there were significant interactions:

a. at 5 and 10 minutes

(1) between 2X and 3X ERA ($F_{1, 14}$=14.923, p<0.01)

(2) between 3X and 4X ERA ($F_{1, 14}$=6.179, p=0.03)

The contrasts show that as the duration of exposure increased (between 5 and 10 minutes), there was greater increase in temperatures as the size of the treatment area decreased.

b. at 10 and 15 minutes

(1) between 2X and 3X ERA ($F_{1, 14}$=27.930, p<0.01)

The contrast shows that as the duration of post-exposure increased (between 10 and 15 minutes) there was greater decrease in temperature at 2X ERA compared with 3X ERA.

c. at 15 and 20 minutes

(1) between 2X and 3X ERA ($F_{1, 14}$=8.125, p=0.01)

The contrast show that as the duration of post-exposure increased (between 15 and 20 minutes), there was greater decrease in temperature at 2X ERA compared with 3X ERA.

All other interactions were not significant (Table 6.2).

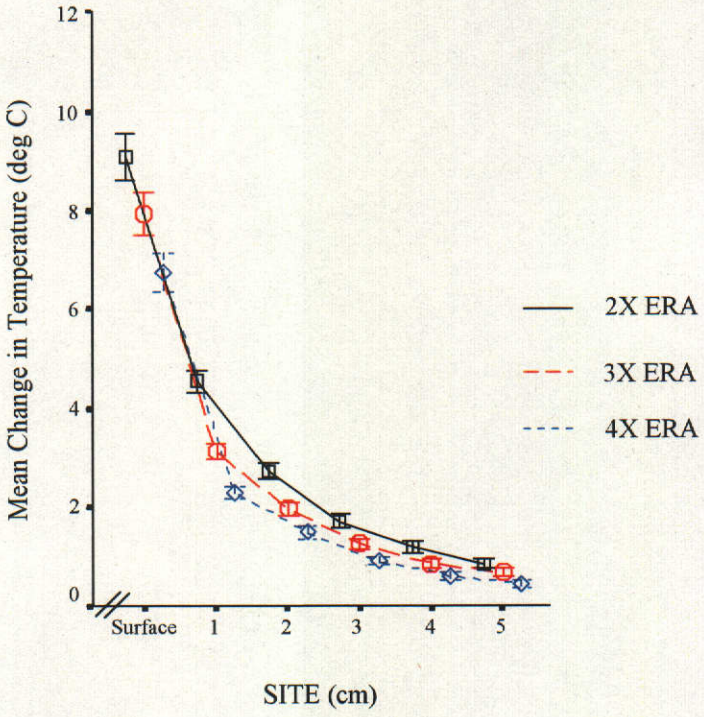

Figure 6.6: Mean (SE) change in temperature for two-way interactions between site (surface, 1, 2, 3, 4, 5 cm) and size of treatment area (2X ERA - small, 3X ERA - medium, and 4X ERA – large).

(2) Size and Site

The results demonstrated that there was a significant 2-way interaction between size of treatment area and site of target tissue on the change in temperature ($F_{4.422,\ 61.911}$=9.801, $p<0.01$; Figure 6.6, Table 6.2). As the size of the treatment area and distance from the surface decreased, there was greater increase in temperature. A post-hoc analysis (repeated contrasts, within subjects, Table 6.2) demonstrated that there were significant interactions:

a. at 2X and 3X ERA

(1) between 1 and 2 cm ($F_{1,\ 14}$=8.708, p=0.01)

(2) between 3 and 4 cm ($F_{1,\ 14}$=5.153, p=0.04)

(3) between 4 and 5 cm ($F_{1,\ 14}$=5.923, p=0.03)

The contrasts show as the size of the treatment area decreased from 3X to 2X ERA, there was greater increase in temperatures between 1 and 2 cm, 3 and 4 cm, and 4 and 5 cm.

b. at 3X and 4X ERA

(1) between 1 and 2 cm ($F_{1, 14}$=6.350, p=0.03)

The contrast shows that as the size of the treatment area decreased from 4X to 3X ERA, there was greater increase in temperatures between 1 and 2 cm.

All other interactions were not significant (Table 6.2).

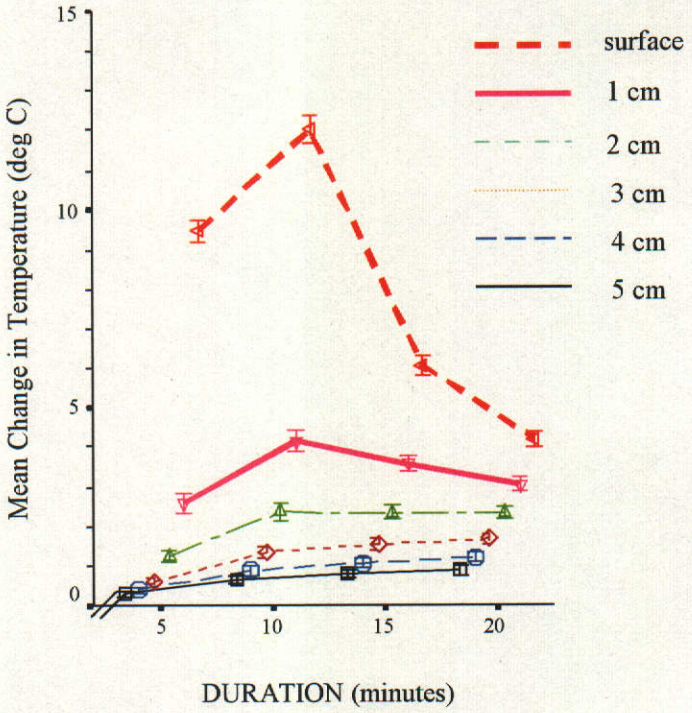

Figure 6.7: Mean (SE) change in temperature for two-way interactions between duration of exposure / post exposure (5, 10, 15 20 minutes) and site (surface, 1, 2, 3, 4, 5 cm).

(3) Duration and Site

The results demonstrated that there was a significant 2-way interaction between duration of exposure and site of target tissue on the change in temperature ($F_{15, 210}$=470.434, $p<0.01$; Figure 6.7, Table 6.2). During the exposure phase (5, 10 minutes), as the distance from the surface decreased and the duration of exposure increased, there was greater increase in temperature. During the post exposure phase, however, two distinct patterns of heating can be seen. For the surface and 1 cm below, as the duration of post-exposure increased, there was a decrease in temperature. For sites greater than 1 cm, however, as the duration of exposure increased, there was a gradual increase in temperature. A post-hoc analysis (repeated contrasts, within subjects, Table 6.2) demonstrated that there were

significant interactions:

a. at 5 and 10 minutes

(1) between surface and 1 cm ($F_{1, 14}$=48.893, $p<0.01$)

(2) between 1 and 2 cm ($F_{1, 14}$=18.915, $p<0.01$)

(3) between 2 and 3 cm ($F_{1, 14}$=48.802, $p<0.01$)

(4) between 3 and 4 cm ($F_{1, 14}$=72.512, $p<0.01$)

(5) between 4 and 5 cm ($F_{1, 14}$=13.049, $p<0.01$)

The contrasts show that as the duration of exposure increased (between 5 and 10 minutes), there was a greater increase in temperature between each adjacent tissue site up to a depth of 5 cm, with the magnitude of this change being greater as the tissues became more superficial.

b. at 10 and 15 minutes

(1) between surface and 1 cm ($F_{1, 14}$=660.524, $p<0.01$)

(2) between 1 and 2 cm ($F_{1, 14}$=18.915, $p<0.01$)

(3) between 2 and 3 cm ($F_{1, 14}$=9.617, $p<0.01$)

The contrasts show that as the duration of post-exposure increased (between 10 and 15 minutes), there was a greater increase in temperature between each adjacent tissue site up to a depth of 3 cm, with the magnitude of this change being greater as the tissues became more superficial.

c. at 15 and 20 minutes

(1) between surface and 1 cm ($F_{1, 14}$=136.485, $p<0.01$)

(2) between 1 and 2 cm ($F_{1, 14}$=77.287, $p<0.01$)

(3) between 2 and 3 cm ($F_{1, 14}$=13.399, $p<0.01$)

(4) between 4 and 5 cm ($F_{1, 14}$=4.525, $p=0.05$)

The contrasts show that as the duration of post-exposure increased (between 15 and 20 minutes), there was a greater increase in temperature between each adjacent tissue site up to a depth of 5 cm (except for between 3 and 4 cm), with the magnitude of this change being greater as the tissues became more superficial.

All other interactions were not significant.

Table 6.2: Repeated Measures ANOVA (General Linear Model, within subjects effects), and Contrasts (within subjects, repeated) for the two-way interaction effects on main factors (Size: 2X, 3X, 4X ERA; Duration: 5, 10, 15, 20 mins; Site: 5, 4, 3, 4, 1, 0 cm).

Factor			df	F value	p value	Eta	Power
Duration	**Size**		4.605	12.855	<0.01	0.479	1.000
5 vs 10	**2X vs 3X**		1	14.923	<0.01	0.516	0.948
	3X vs 4X		1	6.179	0.03	0.306	0.638
10 vs 15	**2X vs 3X**		1	27.930	<0.01	0.666	0.998
	3X vs 4X		1	1.422	0.25	0.092	0.199
15 vs 20	**2X vs 3X**		1	8.125	0.01	0.367	0.755
	3X vs 4X		1	0.007	0.94	0.000	0.051
	Size	**Site**	4.422	9.801	<0.01	0.412	1.000
	2X vs 3X	**5 vs 4**	1	5.923	0.03	0.297	0.620
		4 vs 3	1	5.153	0.04	0.269	0.561
		3 vs 2	1	3.791	0.07	0.213	0.442
		2 vs 1	1	8.708	0.01	0.383	0.783
		1 vs 0	1	0.512	0.49	0.035	0.102
	3X vs 4 X	5 vs 4	1	0.062	0.81	0.004	0.056
		4 vs 3	1	1.556	0.23	0.100	0.214
		3 vs 2	1	0.922	0.35	0.062	0.146
		2 vs 1	1	6.350	0.03	0.312	0.650
		1 vs 0	1	1.171	0.30	0.077	0.172
Duration		**Site**	15	470.434	<0.01	0.971	1.000
5 vs 10		**5 vs 4**	1	13.049	<0.01	0.482	0.919
		4 vs 3	1	72.512	<0.01	0.838	1.000
		3 vs 2	1	48.802	<0.01	0.777	1.000
		2 vs 1	1	18.915	<0.01	0.575	0.981
		1 vs 0	1	48.893	<0.01	0.777	1.000
10 vs 15		5 vs 4	1	0.498	0.49	0.034	0.101
		4 vs 3	1	0.345	0.57	0.024	0.085
		3 vs 2	1	9.617	<0.01	0.407	0.822
		2 vs 1	1	28.535	<0.01	0.671	0.999
		1 vs 0	1	660.524	<0.01	0.979	1.000
15 vs 20		**5 vs 4**	1	4.525	0.05	0.244	0.508
		4 vs 3	1	0.057	0.81	0.004	0.056
		3 vs 2	1	13.399	<0.01	0.489	0.925
		2 vs 1	1	77.287	<0.01	0.847	1.000
		1 vs 0	1	136.485	<0.01	0.907	1.000

df = degrees of freedom; Eta = Estimated effect size (**Note: Bold fonts indicate significant effects or interactions)**

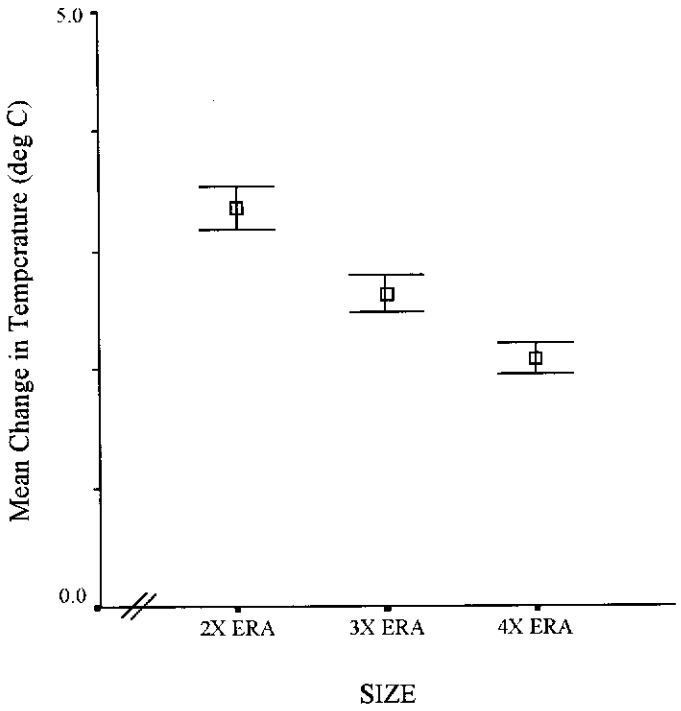

Figure 6.8: Mean (SE) change in temperature for main effect of size of treatment area (2X ERA - small, 3X ERA - medium, and 4X ERA – large).

d. Results of analysis for main factors

(1) Size of treatment area

The results demonstrated that varying the size of the treatment area had a significant effect on the change in temperature ($F_{2, 28}$=21.396, $p<0.01$; Figure 6.8, Table 6.3). A post-hoc analysis (repeated contrasts, within subjects, Table 6.3) demonstrated that there were significant interactions:

a. between 2X and 3X ERA ($F_{1, 14}$=11.365, p=0.01)

b. between 3X and 4X ERA ($F_{1, 14}$=12.838, $p<0.01$)

The contrasts show that as the size of the treatment area decreased from 4X to 3X, and from 3X to 2X ERA, there was greater increase in temperature.

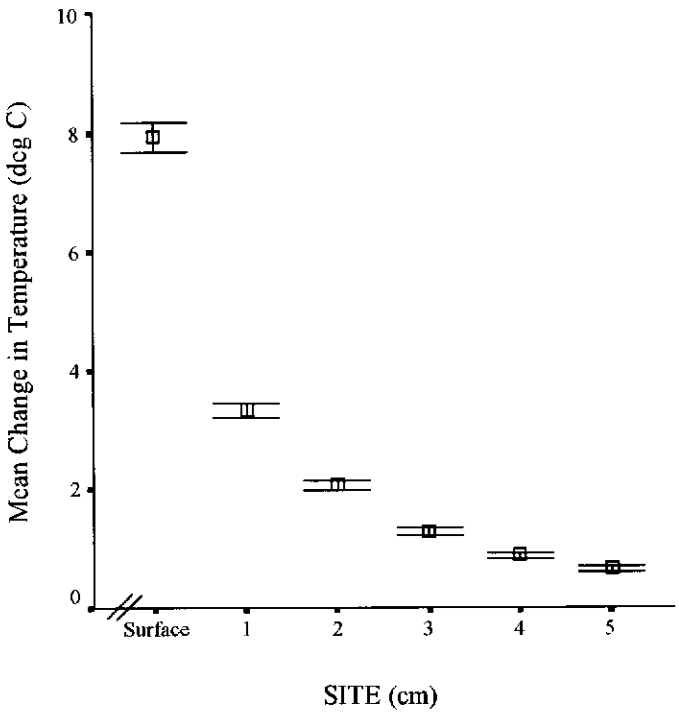

Figure 6.9: Mean (SE) change in temperature for main effect of site of target tissue (surface, 1, 2, 3, 4, 5 cm).

(2) Site of target tissue

The results demonstrated that varying the site of the target tissue had a significant effect on the change in temperature ($F_{2.161, 30.250}$=397.614, $p<0.01$; Figure 6.9, Table 6.3). A post-hoc analysis (repeated contrasts, within subjects, Table 6.3) demonstrated that there were significant interactions:

a. between surface and 1 cm ($F_{1, 14}$=250.251, $p<0.01$)

b. between 1 and 2 cm ($F_{1, 14}$=244.091, $p<0.01$)

c. between 2 and 3 cm ($F_{1, 14}$=41.235, $p<0.01$)

d. between 3 and 4 cm ($F_{1, 14}$=106.070, $p<0.01$)

e. between 4 and 5 cm ($F_{1, 14}$=28.798, $p<0.01$)

The contrasts show that as the distance from the surface decreased (from 5 cm to surface), there was a greater increase in temperature.

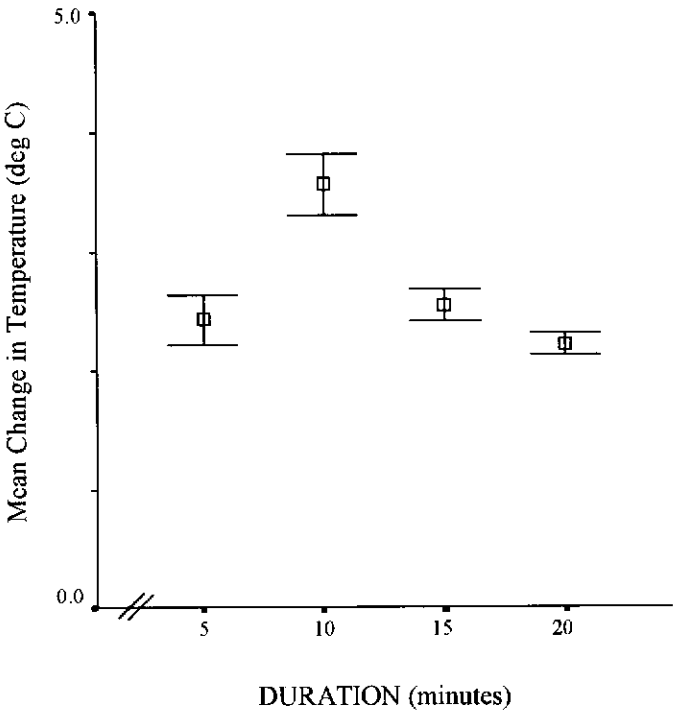

Figure 6.10: Mean (SE) change in temperature for main effect of duration of exposure (5, 10 minutes) and duration of post exposure (15, 20 minutes).

(3) Duration of exposure / post exposure

The results demonstrated that varying the duration of exposure / post exposure had a significant effect on the change in temperature ($F_{2.484, 34.777}$=273.371, $p<0.01$; Figure 6.9, Table 6.3). A post-hoc analysis (repeated contrasts, within subjects, Table 6.3) demonstrated that there were significant interactions:

a. between 5 and 10 minutes ($F_{1, 14}$=351.051, $p<0.01$)

b. between 10 and 15 minutes ($F_{1, 14}$=406.466, $p<0.01$)

c. between 15 and 20 minutes ($F_{1, 14}$=195.622, $p<0.01$)

The contrasts show that as the duration of exposure increased (between 5 and 10 minutes) there was a greater increase in temperature. Conversely, as the duration of post-exposure increased (between 10 and 15 minutes, and between 15 and 20 minutes), there was a greater decrease in temperature.

Table 6.3: Repeated Measures ANOVA (General Linear Model, within subjects effects), and Contrasts (within subjects, repeated) for the main effects on size (2X, 3X, 4X ERA), duration (5, 10, 15, 20 mins) and site (5, 4, 3, 4, 1, 0 cm).

Factor	df	F value	p value	Eta	Power
Size	2	21.396	<0.01	0.604	1.000
2X vs 3X	1	11.365	0.01	0.448	0.880
3X vs 4X	1	12.838	<0.01	0.478	0.914
Site	2.161	397.614	<0.01	0.966	1.000
5 vs 4	1	28.798	<0.01	0.673	0.999
4 vs 3	1	106.070	<0.01	0.883	1.000
3 vs 2	1	41.235	<0.01	0.747	1.000
2 vs 1	1	244.091	<0.01	0.946	1.000
1 vs 0	1	250.251	<0.01	0.947	1.000
Duration	2.484	273.371	<0.01	0.951	1.000
5 vs 10	1	351.051	<0.01	0.962	1.000
10 vs 15	1	406.466	<0.01	0.967	1.000
15 vs 20	1	195.622	<0.01	0.933	1.000

df = degrees of freedom; Eta = Estimated effect size **(Note: Bold fonts indicate significant effects or interactions)**

e. Other results

The means and standard deviations for temperature increases at the skin surface and 1 cm (Table A5-1), 2 cm and 3 cm (Table A5-2), and 4 cm and 5 cm (Table A5-3) below the skin are included in Appendix 5. The thermographic isothermic scans of specimens after 10 minutes exposure and 5 minutes post-exposure to a 1 MHz ultrasound are also included (Figure A5-1, Appendix 5).

6.5 DISCUSSION

a. General

Figures 6.3 illustrate the change in temperatures during the exposure phase (1 to 10 minutes) and the post-exposure phase (11 to 20 minutes) for 1 MHz at the three sizes of treatment area (small or 2X ERA, medium or 3X ERA, and large or 4X ERA). The results of this study for the small sized treatment area (2X ERA) at 1 MHz frequency (Figure 6.3) were very similar to the reliability study (Chapter 4) and

the study on the effect of varying the movement speed of the transducer (Chapter 5), at the same treatment size (2X ERA), movement speed of the transducer (120 beats/min) and frequency of the transducer (1 MHz), in terms of the increase in temperatures, as well as the pattern of heating and cooling obtained at the various tissue sites (see Figure 4.1 in Chapter 4, p107, and Figure 5.2 in Chapter 5, p126). This has been previously described in Chapter 4 (pp 112-3), as well as in Chapter 5 (pp 147-9). In summary, the change in temperature increased as the duration of exposure increased, up to the final 10^{th} minute, and this magnitude of increase was related to the depth of the target tissue, with peak temperatures decreasing as the depth increased (Figure 6.3). For the post-exposure phase, there were two discernible patterns for the superficial tissues (surface, 1 and 2 cm below surface) and for the deep tissues (3, 4 and 5 cm below surface). At the skin surface and at 1 cm below the surface, there was a progressive decrease of the temperatures (Figure 6.3). At 2 cm below surface, the temperature reached a plateau near the peak temperature, and remained relatively constant throughout the entire post-exposure phase (Figure 6.3). For the deep tissues at 3, 4 and 5 cm below the skin surface, there was a gradual increase in temperature (less than 1°C) over the entire 10 minutes post-exposure phase (Figure 6.3). The similar results obtained from all three studies suggests that the heating pattern and the degree of heating observed at all tissue sites at 1.0 Watt/cm^2 could be considered as consistent.

Figure 6.3 also illustrates that while the heating pattern was similar for all three sizes of treatment area, the peak temperatures obtained at all tissue sites were markedly different. This will be elaborated further in the discussion below.

b. Size of treatment area and its interactions

The main aim of this study was to examine the relationship between size of

treatment area and site of the target tissue and duration of exposure / post-exposure. The results demonstrate that the three-way and two-way interactions, and main effects for size of treatment area were all significant. The significant three-way interactions were more obvious between 2X and 3X ERA, and less obvious between 3X and 4X ERA. Smaller treatment sizes appeared to produce greater changes in temperatures at all tissue sites. However, as the size increased from 2X to 3X to 4X ERA, the difference in change in temperatures was not linear. The biggest difference appeared to be between the small (2X ERA) and the other two sizes, while the difference between the medium (3X ERA) and the large (4X ERA) treatment sizes were less pronounced.

The difference in the amount of heating for each of the treatment sizes could be attributed to the greater concentration of ultrasonic energy at smaller sizes of treatment area, which could subsequently be transmitted through the tissues. Larger treatment areas would result in greater dispersion of the energy, hence, reducing the amount of energy, which could be subsequently transmitted through the tissues. The results of this study, therefore, would suggest that more effective heating of the surface and subcutaneous tissues is possible with smaller rather than larger treatment areas.

The clinical implication for this study is that for any course of ultrasonic treatment to be consistent, it is necessary to standardise the size of the treatment area. In other words, even if the other parameters such as output intensity, frequency, and duration of exposure were unchanged, the amount of heating at the target tissue can still vary from session to session if the size of the treatment area is not standardised. From the perspective of a clinical trial, it is even more vital to specify and standardise the size of the treatment area in order to minimize variations in the amount of heating achieved at the target tissue. Unless this is reported correctly in

any clinical trial, the standardization of the treatment area cannot be assumed, and the confounding effect of a non-standardised size of treatment area on the results of any study cannot be discounted.

c. Other results

The results for the other two-way interactions (duration and site), and the one-way effects of site and duration of exposure / post-exposure on change in temperatures were all significant and similar to the results obtained in the study on speed of movement of the transducer (Chapter 5). At 1.0 Watt/cm^2, the change in temperature was dependent on the duration of exposure, as well as the site of the target tissue. As the duration of exposure increased, and as the distance from the skin surface decreased, there is a progressive increase in change in temperature. Conversely, as the duration of post-exposure and the distance from the skin surface increased, there was a progressive decrease in change in temperature. While these relationships were not unexpected and had been demonstrated by the results of this study and the study on movement speed of the transducer (Chapter 5) for 1.0 Watt/cm^2, whether this relationship holds true for the other intensities is unknown and will be investigated in the main study (Chapter 8).

6.6 CONCLUSION

The main results of this study demonstrated that varying the size of the treatment area (2X, 3X and 4X ERA) significantly affected the temperature increase in the target tissues at all tissue depths. Small treatment sizes (2X ERA) produced the greatest depth of heating. Conversely, large treatment sizes (4X ERA) produced the least depth of heating. Therefore, all of the null hypotheses for this study were rejected.

CHAPTER SEVEN

A QUALITATIVE HISTOLOGICAL ANALYSIS OF THE EFFECT OF VARYING THE OUTPUT INTENSITY ON CELL VIABILITY AND THERMAL DAMAGE IN POST-MORTEM PIG TISSUES EXPOSED TO THERAPEUTIC ULTRASOUND

7.1 INTRODUCTION

While there have been few documented cases of adverse reactions to treatment with therapeutic ultrasound (Kitchen 2000), the application of therapeutic ultrasound is not without some risks. Textbooks such as Low and Reed (2000) generally compile lists of conditions and situations where the application of therapeutic ultrasound is contraindicated and these include application over the eyes, in the presence of defective thermal sensation, over tumors, and over the pregnant uterus, among others (Low and Reed 2000). Not all the contraindications, however, are based on experimental evidence, and many have never been confirmed.

MacDonald and Shipster (1981) cautioned that there is insufficient information about the temperatures reached in different tissues, and the extent to which these temperatures are capable of damaging tissues. To date, there have been no studies verifying the claim by MacDonald and Shipster (1981) regarding damage to tissues caused by high temperatures from exposure to various intensities of

therapeutic ultrasound.

While it is reasonable to expect high temperatures could cause tissue damage, the exact nature of damage that can occur is unclear. Because of the high temperatures, thermal injury such as first, second or even third degree burns in the epidermis, dermis, subcutaneous tissues and the adjacent muscle can be expected. However, this has not been demonstrated to occur clinically in a patient population, although experimentally, burns have been found in dogs that have not had their hair coat shaved (Steiss et al, 1999). While it is also possible that other forms of reversible tissue injury may occur as a result of exposure to high intensity therapeutic ultrasound, irreversible thermal injuries are likely to represent the extreme limit of tissue damage. This issue needs to be addressed to establish the range of intensities that can be considered to be outside the safety limits for both clinicians and researchers.

A simple way to address this issue would be to expose tissues to various intensities of ultrasound *in vivo*, and subsequently harvest the tissue for histopathology. However, there are ethical issues associated with tissue harvest and application of damaging doses of ultrasound is likely to be associated with discomfort for the animal.

An alternative method is to expose post-mortem tissues to varying intensities of therapeutic ultrasound *in vitro*. It has been demonstrated that cell death does not occur immediately upon death of the animal and that cells can remain viable for several hours after death of the animal (Yu et al 1990, Hagenah et al 1993, Babapulle and Jayasundera 1993, D'Armini et al 1995, Hirel et al 1996, Gaudin et al 1996, Jones et al 1997, Songsasen et al 1998, Kuang et al 1998, Laywell et al 1999, Tumanov et al 1999, Huang et al 2000,Verwer et al 2002). While the viability of post-mortem cells is somewhat controversial, it is generally accepted that cell

autolysis begins immediately after death of the animal, and in any tissue, the percentage of viable cells versus non-viable cells is a function of time. Post-mortem cell viability has been shown in corneal cells to last from 32 to 50 hours (Hagenah et al 1993, Gaudin et al 1996, Huang et al 2000); in pulmonary cells to last from 1 to 4 hours (D'Armini et al 1995, Jones et al 1997, Kuang et al 1998); in brain cells to last from 4 to 8 hours (Tumanov et al 1999, Verwer et al 2002) and even up to 30 hours (Laywell et al 1999); in spermatozoa to last up to 24 hours (Songsasen et al 1998); in aortic endothelial cells to last from 6 to 8 hours (Yu et al 1990); in blood cells to last from 60 to 84 hours (Babapulle and Jayasundera 1993), and in skin cells to last up to 24 hours (Hirel et al 1996). Therefore, a reasonable time "window" to expose post-mortem skin cells to therapeutic ultrasound can be assumed up to 2 or 3 hours post-mortem without autolysis being a major confounding variable. Subsequently, the assessment of tissue damage from high temperatures following exposure to therapeutic ultrasound can be assessed using standard histological techniques.

The purpose of this qualitative histological study was to investigate the effects of varying the output intensity on irreversible thermal cell injury in post-mortem pig tissues exposed to therapeutic ultrasound.

7.2 NULL HYPOTHESES

The null hypotheses for this study were:

1. Compared with a controlled unexposed specimen, irreversible cellular necrosis due to thermal injury does not occur in post-mortem pig tissues exposed for 10 minutes to a 3 MHz therapeutic ultrasound at output intensities of:
   a. 0.5 Watts/cm^2
   b. 1.0 Watts/cm^2

c. 1.5 Watts/cm^2

2. Compared with a controlled unexposed specimen, irreversible cellular necrosis due to thermal injury does not occur in post-mortem pig tissues exposed for 10 minutes to a 1 MHz therapeutic ultrasound at output intensities of:

a. 0.5 Watts/cm^2

b. 1.0 Watts/cm^2

c. 1.5 Watts/cm^2

d. 2.0 Watts/cm^2

7.3 METHODS

a. Specimens

Eight tissue specimens were obtained from one adult domestic pig weighing approximately 80 kilograms. The eight tissue specimens were allocated for each of the eight intensities being investigated, as follows:

(1) 1 MHz: One specimen each for 0.5, 1.0, 1.5 and 2.0 Watts/cm^2

(2) 3 MHz: One specimen each for 0.0 (control), 0.5, 1.5 and 2.0 Watts/cm^2

The tissue specimens were obtained immediately after slaughter of the animal. Special arrangements were made with the abattoir supplying the specimen to contact the investigator prior to slaughter of the animal and for collection of the specimen immediately after preparations at the abattoir were completed. At no time were the tissues refrigerated. All experiments were completed within two hours of the death of the animal. The left thigh and shoulder of the pig were used for experiments at 1 MHz frequency, while the right thigh and shoulder of the pig were used for experiments at 3 MHz frequency. The controlled unexposed specimen was obtained from the right thigh.

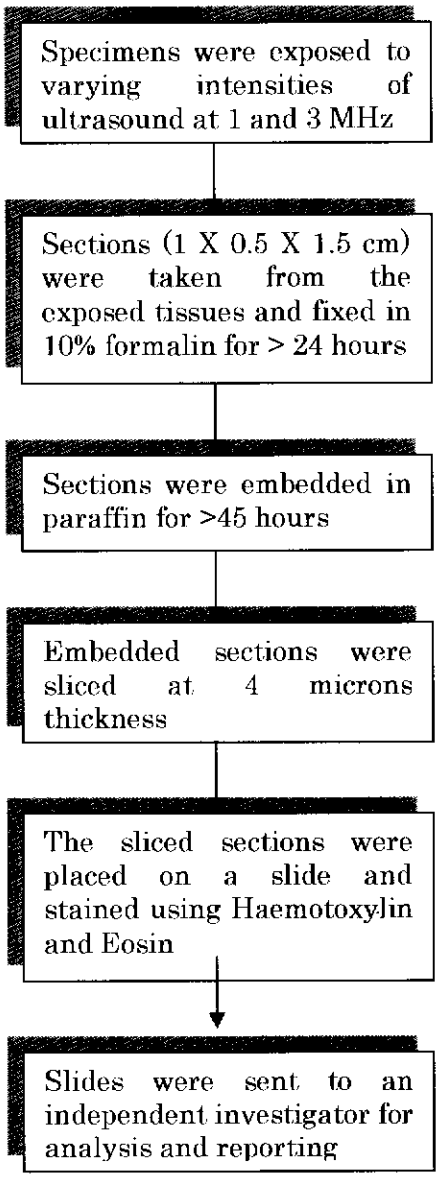

Figure 7.1: Summary of the various stages for preparation of the tissue specimens into histological slides

b. Procedure and Instrumentation

The procedure is summarized in Figure 7.1. Since time was a limiting factor, a research assistant was employed to assist the main investigator. The research assistant applied ultrasound at 1 and 3 MHz for 10 minutes at seven intensities as follows

a. 1 MHz : 0.5, 1.0, 1.5 and 2.0 Watts/cm^2

b. 3 MHz: 0.5, 1.0. 1.5 Watts/cm^2

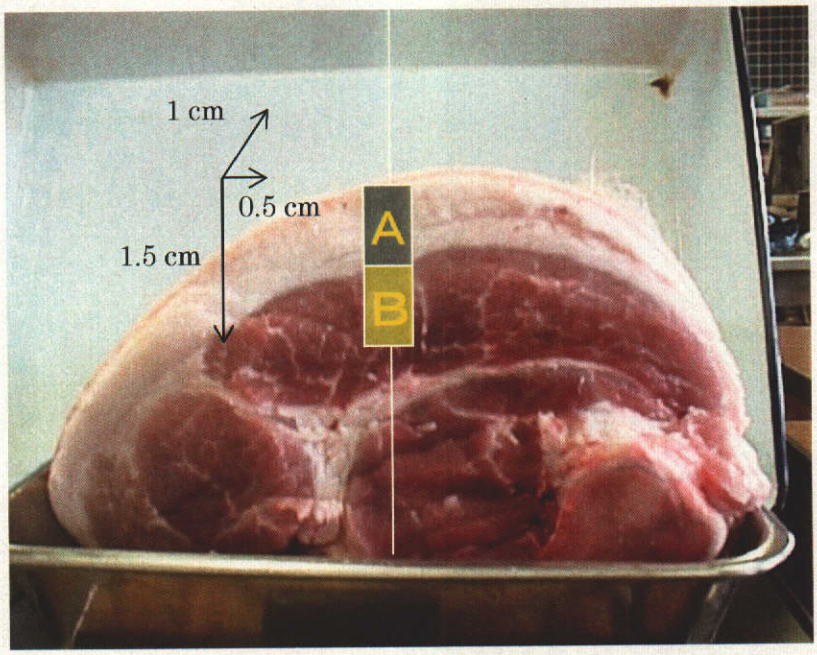

Figure 7.2: Tissue sections (length 1 cm by breadth 0.5 cm by height 1.5 cm) from the specimen were taken immediately after insonation. Portion A consists of skin and fat, while portion B consists of muscle tissues.

The maximum intensities chosen for both 1 and 3 MHz frequencies corresponded to the maximum machine allowable intensities. An unexposed specimen (0.0 Watts/cm^2) served as control. Therapeutic ultrasound at the selected frequency and intensity was applied to the tissues for 10 minutes and the order of selection was randomized. Each selected frequency and intensity was applied to a fresh specimen. The movement speed of the transducer was standardized at 120 beats/minute (moderate speed) and maintained using a metronome. The size of the treatment area was standardized at twice the size of the transducer head (2X ERA) maintained using a thermoplastic guide (see Figure 6.1, Chapter 6, p155), which was secured over the shaved skin of the designated treatment area. A direct in-contact moving soundhead technique using ultrasonic gel was applied.

Immediately following exposure to ultrasound, the specimen was sectioned. Tissue sections (length 1 cm by breadth 0.5 cm by height 1.5 cm) were made at the center of the treatment area as shown in Figure 7.2. The tissue sections were further

divided into two portions corresponding to the skin and fat (portion A), and the muscle (portion B) tissues. Each of these portions was placed in a bottle containing 10% formalin solution in order to fix the tissues prior to embedding, sectioning and staining. The eight specimens (16 portions) were left in the formalin solution for at least 24 hours.

At the end of the experiments, the specimens were disposed of according to the procedure described in Chapter 3 (section 3.4, paragraph j, p100).

The embedding, sectioning and staining procedures were carried out according to standard practices as described by Presnell and Schreibman (1997). Briefly, the tissues were removed from the 10% formalin solution and rinsed under running tap water. Each marked tissue portion was placed in a porous cloth bag and embedded in paraffin using an auto-embedder (Sakura ETP 120-A, Japan). The entire paraffin embedding procedure took 45 hours to complete. Following this, the embedded specimens were removed from their cloth bags and placed in a metal cup with the side to be sectioned facing downwards in the cup. Liquid paraffin was poured into the cup and the allowed to cool, thus forming a solid block with the embedded specimen inside. The cooled block was removed and excess paraffin was trimmed off before mounting the blocks on a microtome (TU213, Yamato Kohki, Japan) for sectioning at 4 microns thickness. The sections were transferred to a glass slide and stained with Haemotoxylin and Eosin according to standard staining procedure (Presnell and Schreibman 1997).

The slides were then sent to an independent, blind investigator for qualitative analysis and reporting of histopathological findings. The independent investigator was a veterinary pathologist with 6 years experience. Slides were coded such that the reporting veterinary pathologist was unaware of the intensities used for all the slides, including the control specimen. The veterinary pathologist

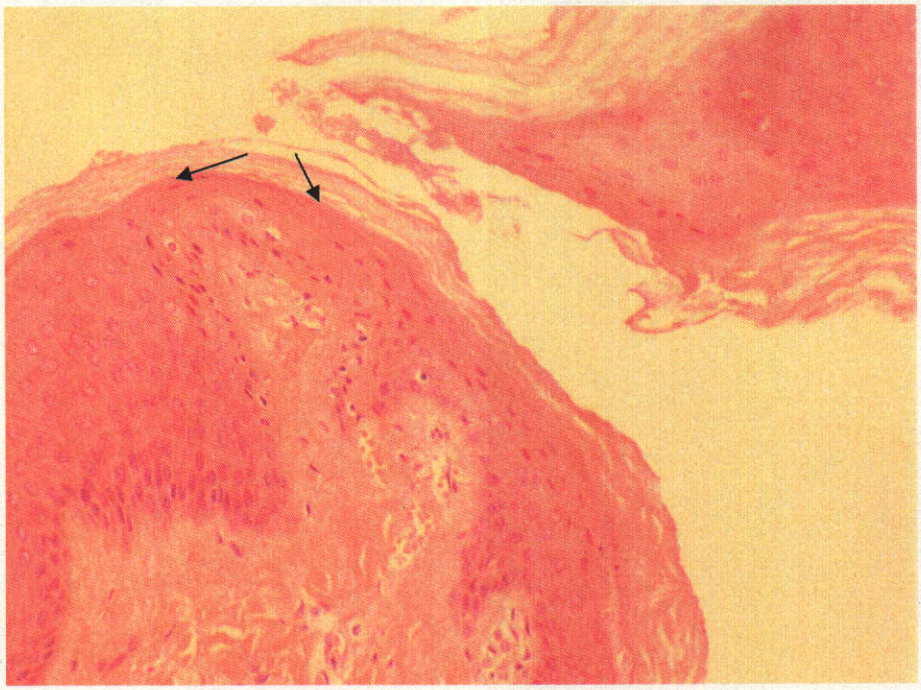

Figure 7.3: Porcine skin tissue exposed for 10 minutes to 2.0 Watts/cm^2 (1MHz) therapeutic ultrasound showing a focal area of epidermal necrosis (area within arrows) (200X magnification).

reported on the amount of observed pathological and thermal damage to the cells in the epidermis, dermis (collagen and vessels), adnexae, panniculus adiposus, and the striated muscles.

7.4 RESULTS

The detailed histopathological findings and comments from the veterinary pathologist are given in Appendix 6.

a. Portion A sections:

Portion A consisted of the epidermis, dermis (collagen and vessels), the adnexae (hair follicles, sweat glands and sebaceous glands), and the panniculus adiposus. Apart from differences in the degree of vascularity between the superficial and mid-dermal plexus, there were minimal differences among the various sections. However, the section marked 8A

(1MHz, 2.0 Watts/cm^2) demonstrated a focal area of epidermal necrosis (see Figure 7.3), which was absent from all the other sections, including the control specimen.

b. Portion B sections:

Portion B consisted mainly of the striated muscle. There were no significant changes among the various sections.

7.5 DISCUSSION

The tissue's response to exposure to ultrasonic energy for *in vivo* cells and viable post-mortem cells cannot be considered to be the same. In *in vivo* cells, physiological processes such as homeostasis, inflammatory and immunological responses are still intact and together, they form the collective response to any external stimuli (Prystowsky and Harber 1994). In post-mortem but viable cells, however, these physiological processes are absent, and hence, any response to external stimuli cannot be considered as typical. In the absence of any physiological defenses, the response by post-mortem viable cells can only be considered as the worst-case scenario. Nevertheless, tissue response from post-mortem viable cells to any stimulus can be considered as part of the response, albeit not the total response seen in *in vivo* cells. On the other hand, in *in vivo* cells, the inflammatory and immunological responses, and to a certain extent the homeostatic response, are not immediate and usually occur from a few minutes up to a few hours depending on the nature and strength of the stimulus (Prystowsky and Harber 1994). In this study, the cells were fixed immediately following exposure to therapeutic ultrasound, and hence even if these physiological defenses were still intact, it is doubtful that they could have played a major role, as they would not have sufficient time to react to the stimulus.

Cell autolysis, however, is something that occurs immediately upon death of the animal. Although it has been demonstrated that cells can remain viable for several hours after the death of the animal, the percentage of viable cells versus non-viable cells decreases with time (Yu et al 1990, Hagenah et al 1993, Babapulle and Jayasundera 1993, D'Armini et al 1995, Hirel et al 1996, Gaudin et al 1996, Jones et al 1997, Songsasen et al 1998, Kuang et al 1998, Laywell et al 1999, Tumanov et al 1999, Huang et al 2000,Verwer et al 2002). In order to partially account for this factor, a controlled unexposed specimen was included in the histological analysis.

In addition, this was the first time that a "hot" post-mortem animal model had been used to detect tissue damage from exposure to varying intensities of thermal energy. While there was some evidence of tissue damage at the maximum machine allowable intensity of 2.0 Watts/cm^2 (1 MHz), all other tissue specimens were unremarkable. In order to test the validity of the post-mortem animal model to detect tissue damage from thermal injury, a separate but similar study was carried out using microwave radiation (unpublished data). In brief, post-mortem pig tissues were exposed to 50, 100, 150 and 200 Watts of microwave energy, at a distance of 5 cm from the skin surface. These tissues were then fixed, embedded, sectioned and stained in exactly the same manner as the ultrasound study, and the histological slides were sent to the same independent veterinary pathologist for qualitative analysis and reporting. The detailed pathology report for the microwave study is given in Appendix 7. In brief, the tissues exposed to 50 Watts of microwave energy demonstrated signs of first degree burns, and those that were exposed to 100, 150 and 200 Watts demonstrated signs of 2nd to 3rd degree burns (see Appendix 7 and Figure 7.4). The results from the microwave study demonstrated that the post-mortem pig model was sensitive to tissue damage caused by thermal injury.

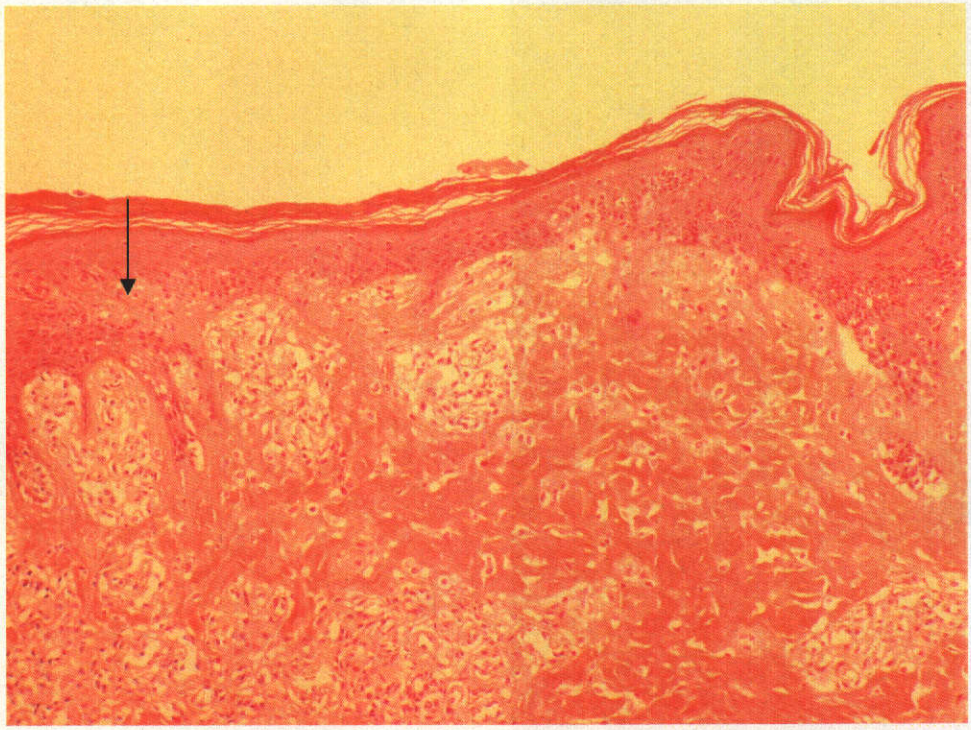

Figure 7.4: Image (100X magnification) of degenerate epidermis and superficial dermis with epidermal clefting and fluid accumulation in the cleft (arrow) in tissues exposed to microwave energy at 150 Watts, suggestive of second to third degree burns.

In contrast to the tissue damage from microwave heating, there was no relationship between ultrasound exposure and tissue damage, except for the 2.0 Watts/cm^2 (1 MHz) intensity. At 2.0 Watts/cm^2 (1 MHz), however, there was evidence of some cellular necrosis at the epidermis. Thermal injury is a possible cause of cellular necrosis and since the epidermis is the site of contact with the ultrasound transducer, it is reasonable to assume that this is the area of skin that will first show signs of any thermal injury. The results of previous studies (Chapter 5 and 6) also clearly demonstrate that the highest increase in temperature from exposure to therapeutic ultrasound occur at the skin's surface. There are, of course, other causes of epidermal necrosis such as chemical injury, autoimmune disease, vascular compromise and some metabolic disorders (Prystowsky and Harber 1994). However, based on the experimental history and lack of any other histological

changes, the epidermal necrosis is most likely attributable to the high temperatures within the tissues caused by exposure to therapeutic ultrasound.

7.6 CONCLUSION

Irreversible thermal injury at the epidermis was shown to occur at 2.0 Watts/cm^2 (1 MHz). Therefore, except for null hypothesis 2d, all the null hypotheses were accepted.

CHAPTER EIGHT

THE EFFECT OF VARYING THE FREQUENCY, OUTPUT INTENSITY AND DURATION OF EXPOSURE ON INCREASE IN TISSUE TEMPERATURES AT VARIOUS DEPTHS IN POST-MORTEM PIG TISSUES EXPOSED TO THERAPEUTIC ULTRASOUND

8.1 INTRODUCTION

When clinicians or researchers compute the treatment dosage for an application of therapeutic ultrasound, the selection of three factors (frequency, intensity and duration of exposure) is critical in determining the amount of heating at the target tissues, assuming that a direct contact gel technique is used, and that the tissues exposed to ultrasound consist of soft-tissues only without the presence of bone. While some of the information is available from the literature, most of the studies do not have, as their primary objective, an intention to clarify this relationship (Draper and Sunderland 1993, Draper et al 1993, 1995b, 1998a, Rimington 1994, Myrer et al 2001). These studies have already been discussed in the literature review (Chapter 2) and their results have been summarized in Table 2.1 (for 1 MHz) and 2.2 (for 3 MHz) (see Chapter 2, p37). Collectively these studies do not provide sufficient details from which to formulate any useful guidelines for determining treatment dosages.

The purpose of this main study was to investigate the effect of varying the frequency, output intensity and duration of exposure on temperature increase at various distances from the skin surface in post-mortem pig tissues exposed to therapeutic ultrasound. A secondary purpose was to explore the possibility of formulating a preliminary model for determining treatment dosages, based on the results of this study.

Prior to this main study, certain protocol related studies were undertaken and completed. The reliability of the two instruments used to measure change in temperature (dependent variable) was established initially (Chapter 4). The movement speed of the transducer was recommended at 120 beats/min (Chapter 5). The size of the treatment area was standardized at 2X ERA (Chapter 6). The upper limit of the output intensities to be investigated was recommended at 1.5 Watts/cm^2 for both 1 and 3 MHz frequencies (Chapter 7).

8.2 NULL HYPOTHESES

The null hypotheses for this study were:

1. Varying the frequency of the ultrasound generator, that is (a) 1MHz, or (b) 3 MHz; does not affect the temperature increases in post-mortem pig tissues exposed to therapeutic ultrasound at:
   i. the skin surface,
   ii. 1 cm below skin surface,
   iii. 2 cm below skin surface,
   iv. 3 cm below skin surface,
   v. 4 cm below skin surface,
   vi. 5 cm below skin surface.
2. Varying the output intensity, that is (a) 0.1, (b) 0.3, (c) 0.5, (d) 0.7,

(e) 0.9, (f) 1.1, (g) 1.3, or (h) 1.5 Watts/cm^2; does not affect the temperature increase in post-mortem pig tissues exposed to therapeutic ultrasound at:

i. the skin surface,

ii. 1 cm below skin surface,

iii. 2 cm below skin surface,

iv. 3 cm below skin surface,

v. 4 cm below skin surface,

vi. 5 cm below skin surface.

3. Varying the duration of exposure, that is (a) 5, (b) 10 minutes; and post-exposure (c) 15, (d) 20 minutes; does not affect the temperature increase in post-mortem pig tissues exposed to therapeutic ultrasound at:

i. the skin surface,

ii. 1 cm below skin surface,

iii. 2 cm below skin surface,

iv. 3 cm below skin surface,

v. 4 cm below skin surface,

vi. 5 cm below skin surface.

8.3 METHODS

a. Specimens

Twenty tissue specimens, obtained from adult post-mortem pigs, were processed as previously described (Chapter 3, section 3.4, pp92-94). This experiment entailed testing at 2 frequencies and 8 intensities. Ten specimens were used for 1 MHz applications and the other 10 (matching contralateral side) for 3

MHz applications. Each specimen was used 4 times. For each frequency 5 of the allocated specimens were used for four of the eight intensities and the other 5 specimens for the remaining 4 intensities. The equal division of the eight intensities into 2 groups, and using one specimen for each group, was done in order to avoid denaturing of the tissues due to over-exposure to ultrasonic energy. Specimens were left for at least an hour at room temperature between applications to allow them to return to baseline temperature.

b. Instrumentation and Procedure

Instrumentation and procedures employed were identical to those discussed in Chapter 3 (sections 3.3 and 3.4, pp88-100). The selection of the experimental parameters has been described in Chapter 3 (section 3.4, paragraph i, p100; and Table 3.1, p87), and was based on the findings reported in Chapters 4, 5, 6 and 7.

The procedure for this study is summarized in Figure 8.1. Therapeutic ultrasound at 1 MHz and 3 MHz frequencies was applied to the tissues for 10 minutes at various intensities ranging from 0.1 to 1.5 Watts/cm^2 in a stepwise increment of 0.2 Watts/cm^2 (0.1, 0.3, 0.5, 0.7. 0.9, 1.1, 1.3 and 1.5 Watts/cm^2). Increases in tissue temperatures at six sites (skin surface, and 1, 2, 3, 4 and 5 cm below the skin surface) were recorded at baseline (prior to exposure) and subsequently at one-minute intervals during the 10-minutes exposure (1st to 10th minute), as well as for another 10 minutes post-exposure (11th to 20th minute). Surface skin temperature was recorded with the infrared spot thermometer (Minolta HT-11) while the subcutaneous temperatures (1, 2, 3, 4 and 5 cm below skin surface) were measured with the infrared video thermography unit (Avio TVS 2000).

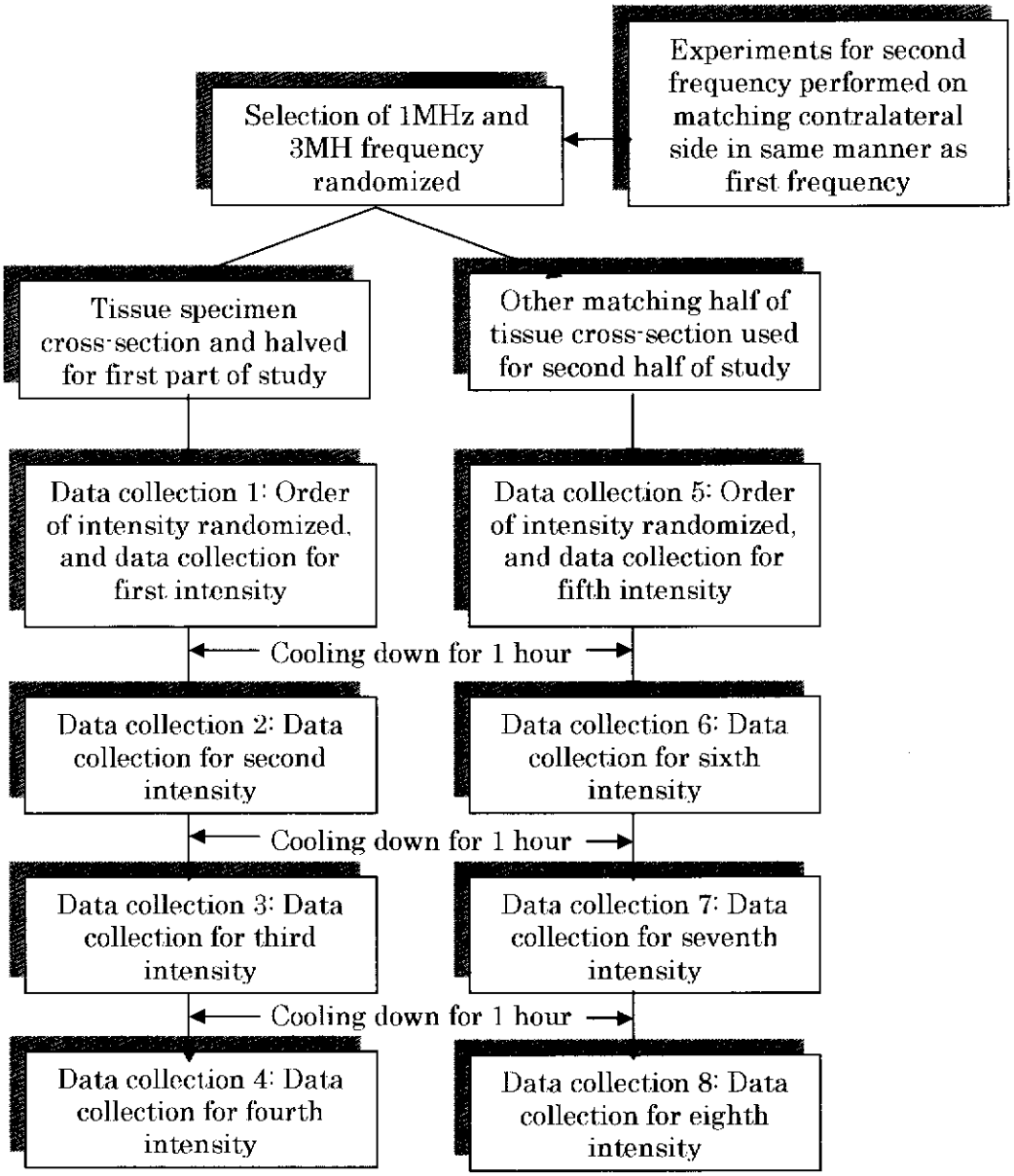

Figure 8.1: Flow-chart of detailed procedure for effect of varying frequency, intensity and duration on the depth of heating

The movement speed of the transducer was standardised at 120 beats/min (moderate speed), and maintained with a metronome. The size of the treatment area was standardised at twice the size of the transducer (2X ERA), and maintained using a thermoplastic guide. A direct in-contact moving soundhead technique using ultrasonic gel was applied.

The order of selection of the two frequencies and eight intensities was randomized. At the end of each 20-minute experiment, the tissue specimen was

allowed to cool down for at least an hour and the experimental setup was left in situ each time. The same investigator performed all the measurements.

c. Data Analysis

A general description for data analysis has been provided in Chapter 3 (section 3.5, p101).

Data from all specimens were analysed. Analysis of the data was performed on mean change in temperature (dependent variable), rather than actual temperature measured, at the 5^{th}, 10^{th}, 15^{th}, and 20^{th} minutes. The reliability at these four time periods was found to be representative of the exposure and post-exposure phases (Chapter 4). However, data for all 20 minutes of sampling are provided in the table of means (Appendix 8) and in Figures 8.2 and 8.3.

A two (frequency – 1 and 3 MHz) by eight (intensities – 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5 Watts/cm^2) by four (duration – 5, 10, 15, 20 minutes) by six (sites – skin surface, and 1, 2, 3, 4, 5 cm below skin surface) repeated measures analysis of variance was used to assess the effect of varying the frequency, intensity and duration of exposure on the increase in tissue temperatures (dependent variable) on the skin surface, as well as at the five tissue depths. For those factors that were found to be significantly different, a repeated contrasts (within subjects) post-hoc analysis on factors that had greater than two levels was performed to identify the differences among the levels.

In addition, the observed power, the estimates of effect size and descriptive statistics for means and standard deviations of the scores were also calculated. The assumptions of sphericity, equal variance and normality of the statistical procedures were checked.

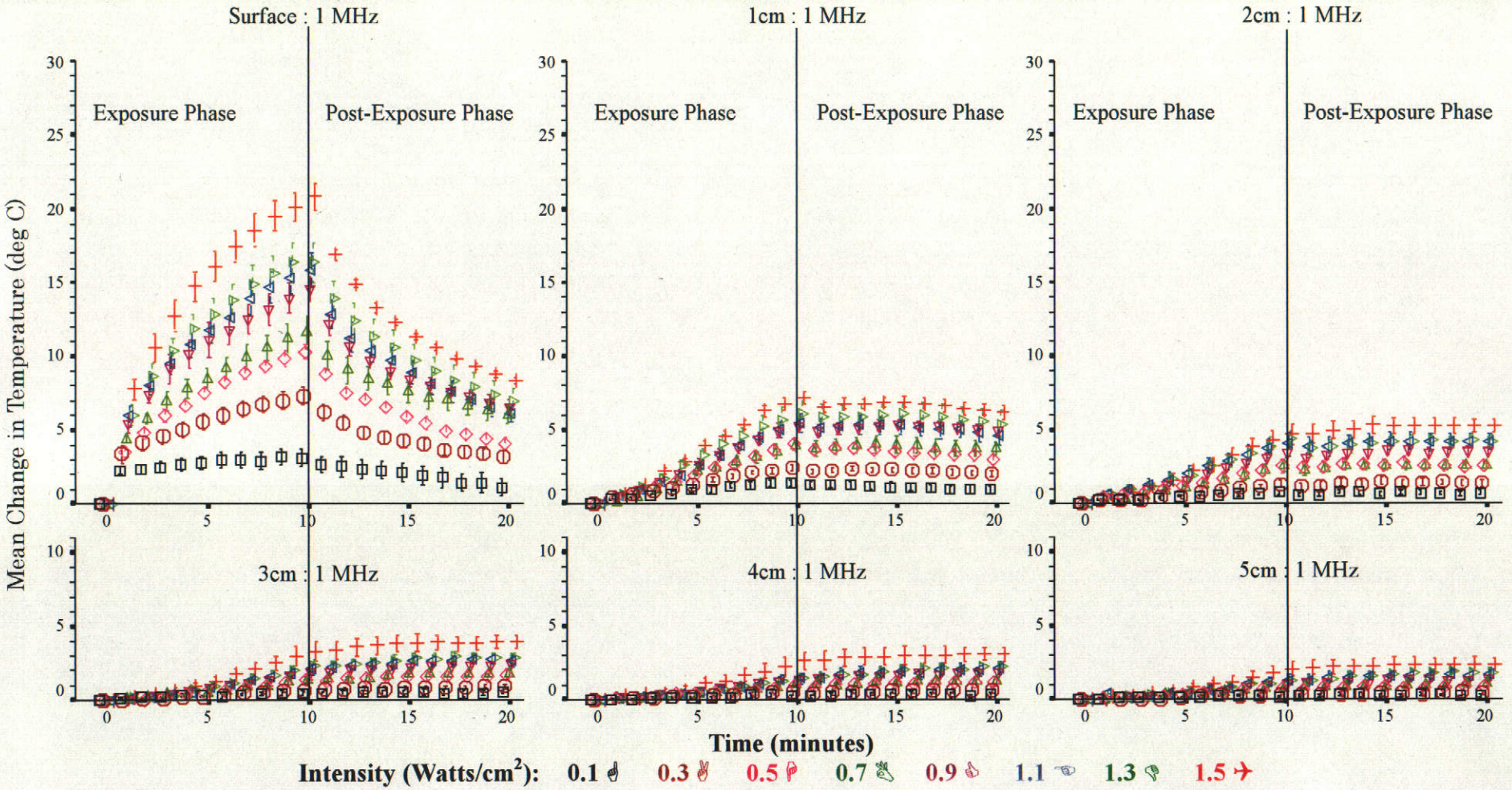

Figure 8.2: Mean increase in temperatures for 10 minutes exposure and 10 minutes post-exposure to therapeutic ultrasound at moderate movement speed of the transducer for small treatment area for 1MHz frequency at 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3 and 1.5 watts/cm^2

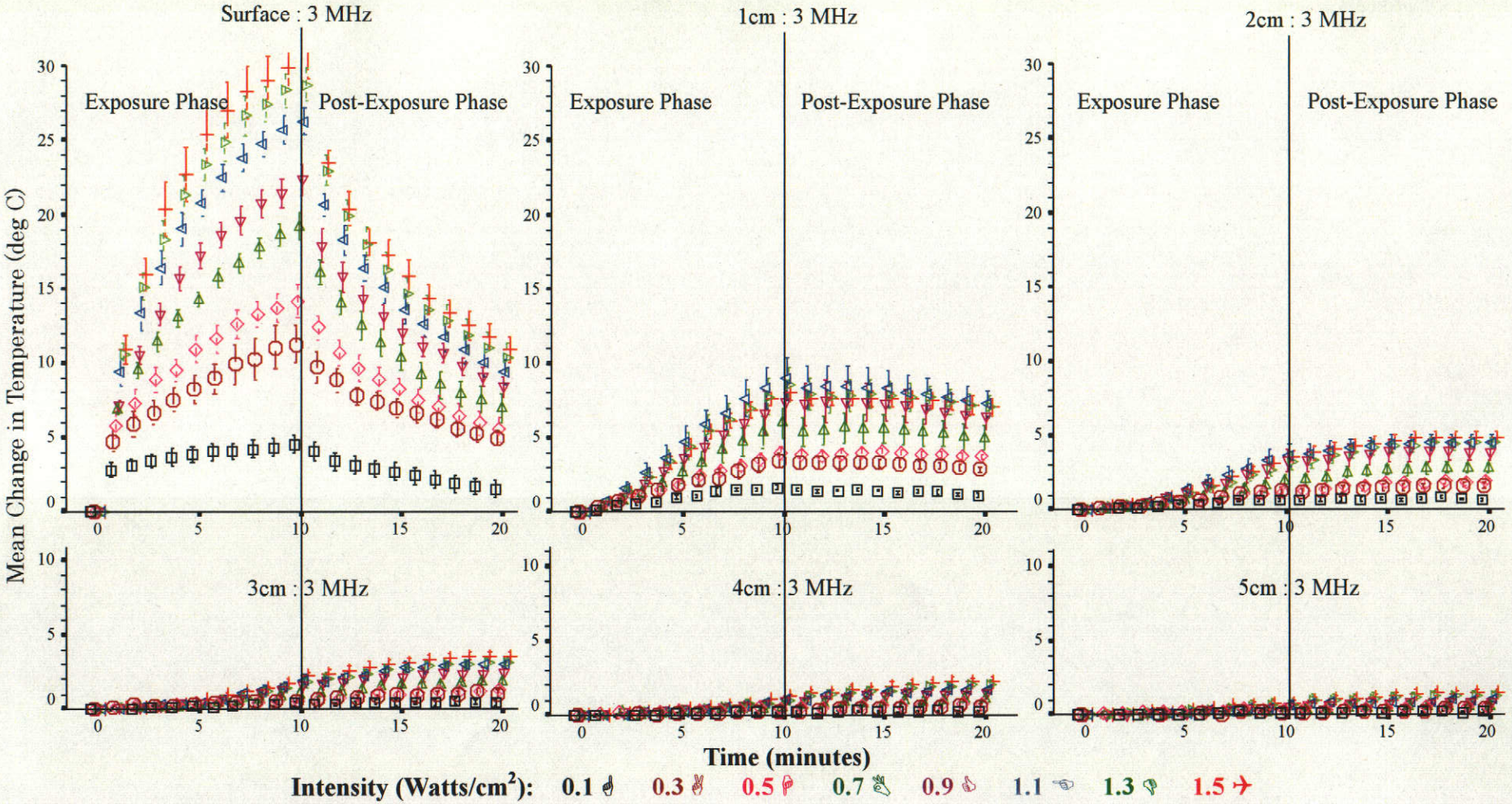

Figure 8.3: Mean increase in temperatures for 10 minutes exposure and 10 minutes post-exposure to therapeutic ultrasound at moderate movement speed of the transducer for small treatment area for 3MHz frequency at 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3 and 1.5 watts/cm^2

Table 8.1: Summary of peak temperatures reached and range from the lowest (0.1Watt/cm^2) to the highest (1.5Watts/cm^2) intensities, at the various tissue sites for both 1 and 3 MHz, after the maximum 10 minutes exposure time

Site	1 MHz			3 MHz		
	0.1W/cm^2 °C	1.5W/cm^2 °C	Range °C	0.1W/cm^2 °C	1.5W/cm^2 °C	Range °C
Surface	3.16	20.86	17.7	4.54	30.86	26.32
1 cm	1.38	7.19	5.81	1.63	8.03	6.40
2 cm	0.70	4.70	4.00	0.64	3.54	2.90
3 cm	0.45	3.36	2.91	0.38	2.23	1.85
4 cm	0.22	2.64	2.42	0.20	1.24	1.04
5 cm	0.16	2.05	1.89	0.12	0.80	0.68

8.4 RESULTS

The main factors of interest in the analysis were frequency, intensity, duration of exposure / post-exposure, site of target tissue and their interactions. A general description of the results will be presented first. The results of the main effects and interactions appear to be cumulative. Hence, to facilitate understanding of the various interactions, the results for the main effects of each of the four factors will be described first, followed by their two-way, three-way and four-way interactions.

a. General

The mean change in temperature over time for the eight intensities (0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3 and 1.5 Watts/cm^2) at the skin surface, and at 1, 2, 3, 4, and 5 cm below the skin is shown in Figures 8.2 and 8.3 for 1 and 3 MHz respectively. A summary of the peak temperatures reached at the various tissue sites for the lowest (0.1 Watt/cm^2) and the highest (1.5 Watt/cm^2) intensities for both 1 and 3 MHz are given in Table 8.1. The largest change in temperature occurred at the skin surface at the 1.5 Watts/cm^2 intensity for both 1 MHz and 3 MHz, which was almost one and a half times greater for 3 MHz (30.86 deg C) than for 1 MHz (20.86 deg C; Figures

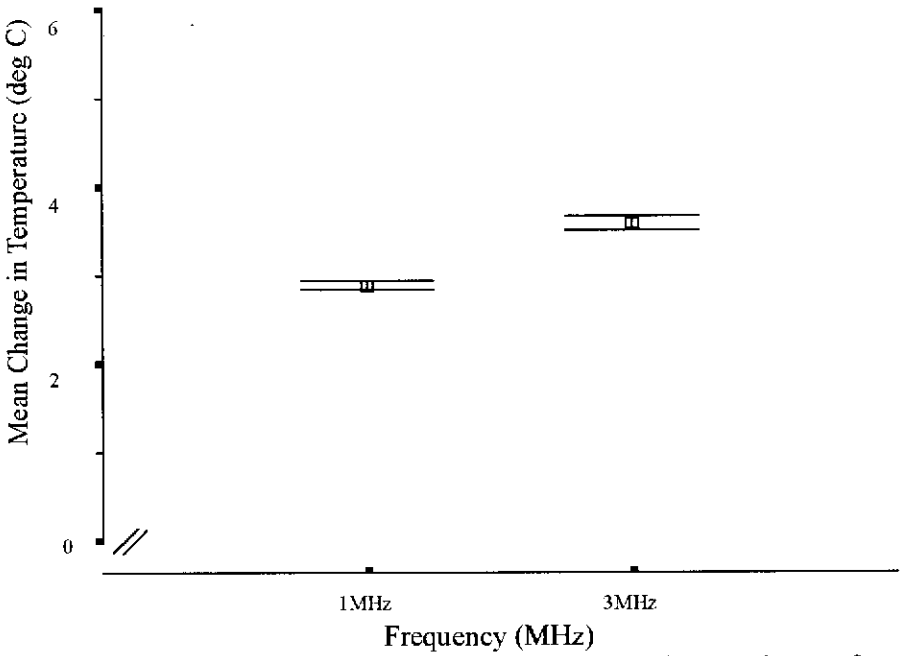

Figure 8.4: Mean (SE) change in temperature in relation to frequency

8.2 and 8.3, Table 8.1). The mean change in temperature decreased as the distance from the surface increased and as the intensity decreased (Figures 8.2 and 8.3), and this was similar for both 1 and 3 MHz. From Table 8.1, it can be seen that the greatest dispersion of scores between the lowest (0.1 Watt/cm^2) and highest (1.5 Watts/cm^2) intensity was at the skin surface with a temperature range of 17.7°C for 1 MHz, and 26.32°C for 3 MHz, at maximum exposure time (10 minutes). As the distance from the skin surface increased, the dispersion of scores between each of the eight intensities decreased for both frequencies (Table 8.1).

b. Results of analysis for main factors

(1) Frequency

Varying the frequency (1 MHz versus 3 MHz) had a significant effect on the mean change in temperature ($F_{1,4}$=8.812, p=0.04) (Table 8.2, Figure 8.4). The mean increase in temperature was greater for the 3 MHz compared with the 1 MHz transducer.

Table 8.2: Repeated Measures ANOVA (General Linear Model, within subjects effects), and Contrasts (within subjects, repeated) for the one-way effects on main factors (Frequency, Intensity, Duration, Site) (Bold fonts indicate significant effects)

Factor			df	F value	p value	Eta	Power
Freq			1	8.812	0.04	0.688	0.611
	Intensity		7	106.977	<0.01	0.964	1.000
	0.1 vs 0.3		1	526.995	<0.01	0.992	1.000
	0.3 v s0.5		1	133.820	<0.01	0.971	1.000
	0.5 vs 0.7		1	11.291	0.03	0.738	0.712
	0.7 vs 0.9		1	13.464	0.02	0.771	0.782
	0.9 vs 1.1		1	1.725	0.26	0.301	0.175
	1.1 vs 1.3		1	0.722	0.44	0.153	0.102
	1.3 vs 1.5		1	5.734	0.08	0.589	0.447
		Duration	3	426.242	<0.01	0.991	1.000
		5 vs 10	1	2111.752	<0.01	0.998	1.000
		10 vs 15	1	707.970	<0.01	0.994	1.000
		15 vs 20	1	560.400	<0.01	0.993	1.000
		Site	5	214.727	<0.01	0.982	1.000
		5 vs 4	1	121.810	<0.01	0.968	1.000
		4 vs 3	1	415.052	<0.01	0.990	1.000
		3 vs 2	1	283.375	<0.01	0.986	1.000
		2 vs 1	1	37.211	<0.01	0.903	0.992
		1 vs 0	1	129.470	<0.01	0.970	1.000

Freq = Frequency; df = degrees of freedom, Eta = Estimated effect size

Table 8.3: Additional post-hoc contrasts (simple contrasts, within subjects) for the one-way effect on intensities at 0.9, 1.1, 1.3 and 1.5 Watts/cm^2 (Bold fonts indicate significant effects)

	0.9 Watts/cm^2	1.1 Watts/cm^2	1.3 Watts/cm^2
1.1 Watts/cm^2	F=1.725; p=0.26		
1.3 Watts/cm^2	F=6.827; p=0.06	F=0.722; p=0.44	
1.5 Watts/cm^2	**F=22.914; p=0.01**	**F=34.357; p<0.01**	F=5.734; p=0.08

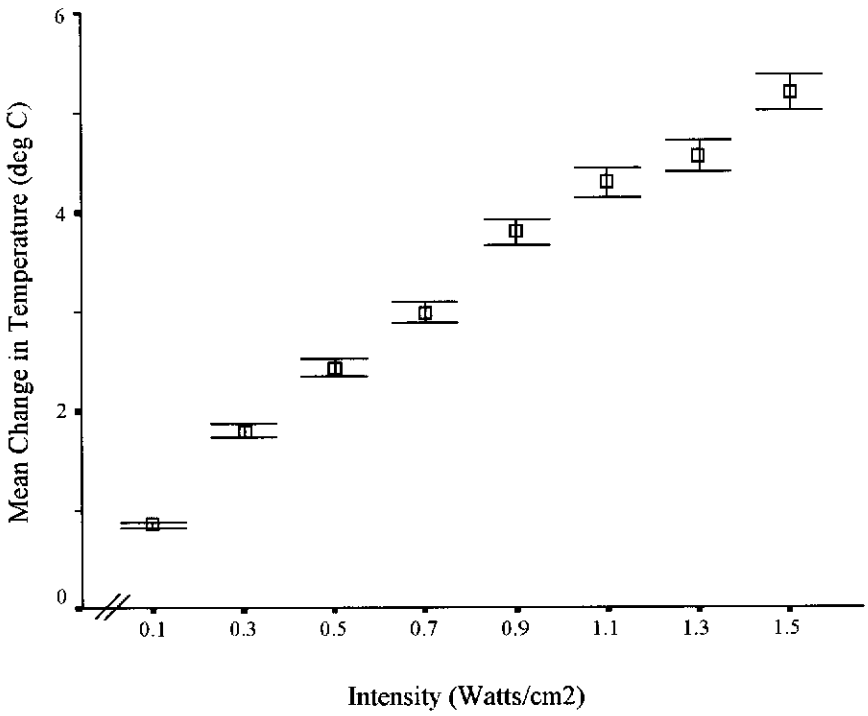

Figure 8.5: Mean (SE) change in temperature in relation to intensity

(2) Intensity

Varying the intensity had a significant effect on the temperature increase ($F_{7, 28}$=0.007, p<0.01, Figure 8.5). As intensity increased, the temperatures increased in an almost linear manner. A post-hoc analysis (repeated contrasts, within subjects, Table 8.2) demonstrated that there were significant differences between:

a. 0.1 and 0.3 Watts/cm^2 ($F_{1, 4}$=526.995, p<0.01);

b. 0.3 and 0.5 Watts/cm^2 ($F_{1, 4}$=133.820, p<0.01);

c. 0.5 and 0.7 Watts/cm^2 ($F_{1, 4}$=11.291, p=0.03);

d. 0.7 and 0.9 Watts/cm^2 ($F_{1, 4}$=13.434, p=0.02).

However, there were no significant differences between:

a. 0.9 and 1.1 Watts/cm^2 ($F_{1, 4}$=1.725, p=0.26);

b. 1.1 and 1.3 Watts/cm^2 ($F_{1, 4}$=0.722, p=0.44);

c. 1.3 and 1.5 Watts/cm^2 ($F_{1, 4}$=5.734, p=0.08).

These contrasts show that at intensities lower than 0.9 Watts/cm^2, there were significant differences in the mean change in temperatures between each of the adjacent intensities. For intensities greater than 0.9 Watts/cm^2 however, the mean change in temperature was not significantly different between each adjacent intensity, up to 1.5 Watts/cm^2. In order to clarify the relationship between the various intensities above 0.9 Watt/cm^2, additional contrasts (simple contrasts, within subjects) were performed and are summarized in Table 8.3.

The results of the additional analysis showed that the mean change in temperatures for 1.5 Watts/cm^2 was significantly different from 0.9 and 1.1 Watts/cm^2, but was not significantly different from 1.3 Watts/cm^2. The analysis also demonstrated that there was no significant difference in the mean change in temperature between 0.9, 1.1 and 1.3 Watts/cm^2 (see Table 8.3).

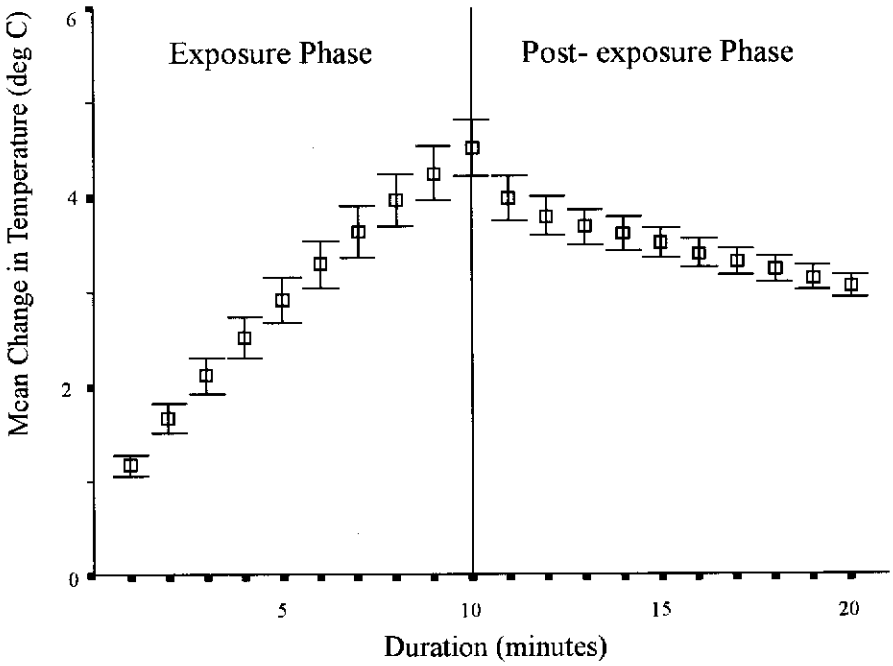

Figure 8.6: Mean (SE) change in temperature in relation to duration of exposure (1 to 10 minutes) and post-exposure (11 to 20 minutes)

(3) Duration of Exposure

Varying the duration of exposure had a significant effect on the mean change in temperature ($F_{3, 12}$=426.242, $p<0.01$, Figure 8.6). As the duration of exposure increased from 1 to 10 minutes, the temperatures increased gradually in an almost linear manner and peaked at 10 minutes (maximum exposure time). During the post-exposure phase, as the duration increased from 11 to 20 minutes, the temperatures decreased but did not return to baseline. A post-hoc analysis (repeated contrasts, within subjects, Table 8.2) showed that there were significant differences between:

a. 5 and 10 minutes ($F_{1, 4}$=2111.752, $p<0.01$);

b. 10 and 15 minutes ($F_{1,4}$=707.970, $p<0.01$);

c. 15 and 20 minutes ($F_{1, 4}$=560.400, $p<0.01$),

d. 0 (baseline) and 20 minutes ($F_{1, 4}$=2788.324, $p<0.01$).

The contrasts showed that the rate of change in temperatures were significantly different between each exposure and post-exposure times.

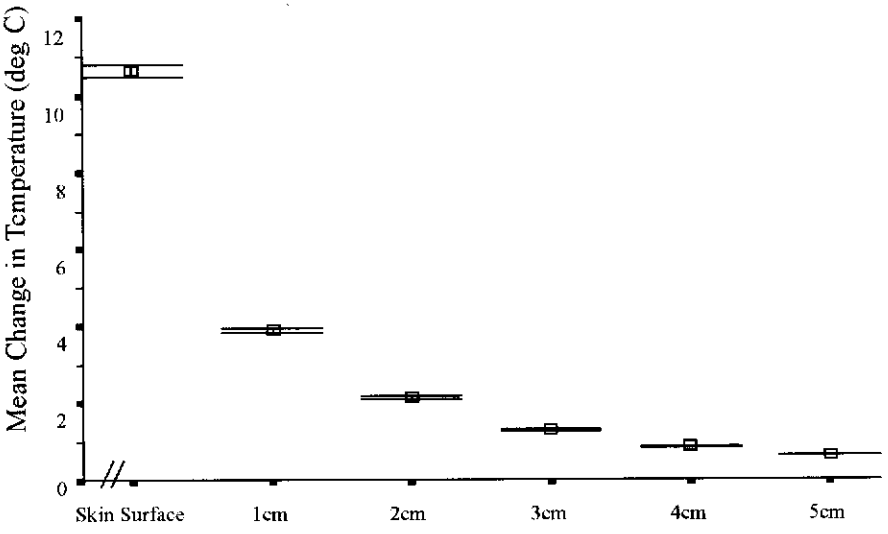

Figure 8.7: Mean (SE) change in temperature in relation to site (distance from skin surface)

(4) Site

Varying the site of the target tissue (distance from skin surface) had a significant effect on the mean change in temperature ($F_{5, 20}$=214.727, $p<0.01$, Figure 8.7). As the distance from the skin surface increased, the mean change in temperature decreased exponentially. A post-hoc analysis (repeated contrasts, within subjects, Table 8.2) demonstrated that there were significant differences between:

a. skin surface and 1 cm ($F_{1, 4}$=129.470, $p<0.01$);

b. 1 cm and 2 cm ($F_{1, 4}$=37.211, $p<0.01$);

c. 2 cm and 3 cm ($F_{1, 4}$=283.375, $p<0.01$);

d. 3 cm and 4 cm ($F_{1, 4}$=415.052, $p<0.01$);

e. 4 cm and 5 cm ($F_{1, 4}$=121.810, $p<0.01$)

The contrasts showed that the mean change in temperature at each adjacent tissue site was significantly different from the site above and below, and decreased exponentially from surface to 5 cm below.

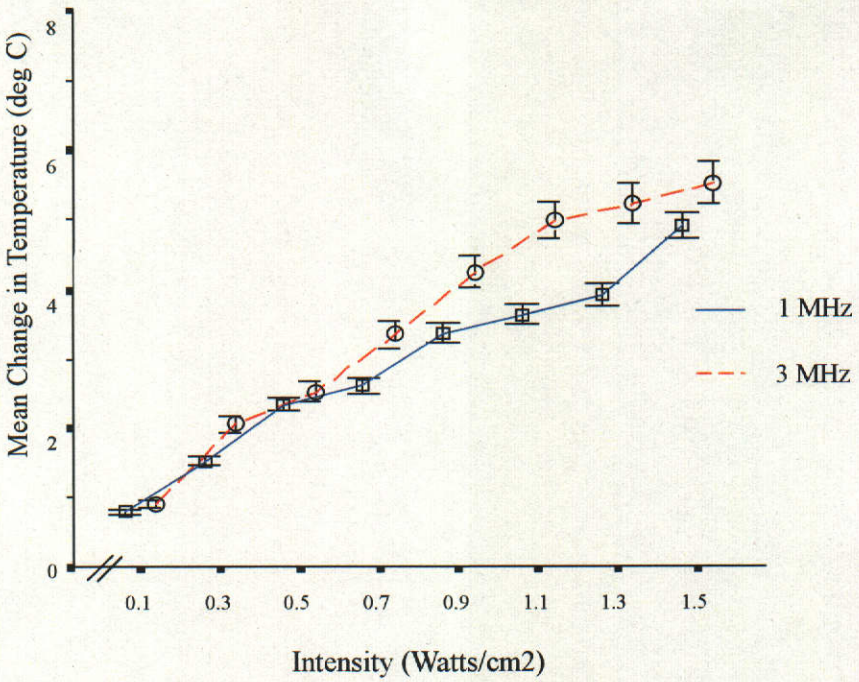

Figure 8.8: Mean (SE) change in temperature for two-way interactions between frequency and intensity

c. Results of analysis for two-way interaction

(1) Frequency and Intensity

There was a significant two-way interaction between frequency and intensity on mean change in temperature ($F_{7, 28}$=3.191, p<0.01, Figure 8.8). A post-hoc analysis (repeated contrasts, within subjects, Table 8.4) demonstrated that there were significant differences between:

a. 1 and 3 MHz

(1) between 0.1 and 0.3 Watts/cm^2 ($F_{1, 4}$=21.913, p=0.01);

(2) between 0.5 and 0.7 Watts/cm^2 ($F_{1, 4}$=13.801, p=0.02).

The contrasts showed that the mean change in temperature between 0.1 and 0.3 Watts/cm^2 was greater for 3 MHz, and conversely, between 0.5 and 0.7 Watts/cm^2 was greater for 1 MHz (see Figure 8.8). No other interactions were significant (Table 8.4).

Table 8.4: Repeated Measures ANOVA (General Linear Model, within subjects effects), and Contrasts (within subjects, repeated) for the two-way interaction effects on main factors (Frequency, Intensity, Duration, Site) (Bold fonts indicate significant interactions)

Factor				df	F value	p value	Eta	Power
Freq X	**Intensity**			7	3.191	0.01	0.444	0.885
1 vs 3	**0.1 vs 0.3**			1	21.913	0.01	0.846	0.930
	0.3 vs 0.5			1	2.895	0.16	0.420	0.260
	0.5 vs 0.7			1	13.801	0.02	0.775	0.791
	0.7 vs 0.9			1	3.818	0.12	0.488	0.324
	0.9 vs 1.1			1	5.367	0.08	0.573	0.424
	1.1 vs 1.3			1	0.048	0.84	0.012	0.053
	1.3 vs 1.5			1	1.378	0.31	0.256	0.150
Freq	**X**	**Duration**		1.321	9.064	0.02	0.694	0.736
1 vs 3		5 vs 10		1	0.980	0.38	0.197	0.121
		10 vs 15		1	130.888	<0.01	0.970	1.000
		15 vs 20		1	9.062	0.04	0.694	0.622
Freq	**X**		**Site**	5	55.543	<0.01	0.933	1.000
1 vs 3			5 vs 4	1	0.374	0.57	0.086	0.077
			4 vs 3	1	2.136	0.22	0.348	0.205
			3 vs 2	1	0.022	0.89	0.006	0.052
			2 vs 1	1	47.947	<0.01	0.923	0.999
			1 vs 0	1	29.817	0.01	0.882	0.977
	Intensity X	**Duration**		21	18.657	<0.01	0.823	1.000
	0.1 vs 0.3	**5 vs 10**		1	352.200	<0.01	0.989	1.000
	0.3 vs 0.5			1	12.388	0.02	0.756	0.749
	0.5 vs 0.7			1	18.575	0.01	0.823	0.889
	0.7 vs 0.9			1	9.908	0.04	0.712	0.659
	0.9 vs 1.1			1	3.560	0.13	0.471	0.306
	1.1 vs 1.3			1	0.027	0.88	0.007	0.052
	1.3 vs 1.5			1	1.432	0.30	0.264	0.154
	0.1 vs 0.3	**10 vs 15**		1	15.731	0.02	0.797	0.838
	0.3 vs 0.5			1	8.593	0.04	0.682	0.600
	0.5 vs 0.7			1	3.441	0.14	0.462	0.298
	0.7 vs 0.9			1	3.367	0.14	0.457	0.293
	0.9 vs 1.1			1	4.631	0.10	0.537	0.378
	1.1 vs 1.3			1	0.128	0.74	0.031	0.059
	1.3 vs 1.5			1	1.371	0.31	0.255	0.149
	0.1 vs 0.3	15 vs 20		1	7.127	0.06	0.641	0.526
	0.3 vs 0.5			1	1.359	0.31	0.254	0.148
	0.5 vs 0.7			1	0.270	0.63	0.063	0.069
	0.7 vs 0.9			1	2.594	0.18	0.393	0.238
	0.9 vs 1.1			1	5.825	0.07	0.593	0.452
	1.1 vs 1.3			1	0.865	0.41	0.178	0.112
	1.3 vs 1.5			1	0.559	0.50	0.123	0.090

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Table 8.4: continued from previous page

Factor				df	F value	p value	Eta	Power
Freq X	Intensity X	DurationX	Site					
	Intensity	X	**Site**	35	52.301	<0.01	0.929	1.000
	0.1 vs 0.3		5 vs 4	1	0.635	0.47	0.137	0.096
			4 vs 3	1	1.117	0.35	0.218	0.131
			3 vs 2	1	10.686	0.03	0.728	0.690
			2 vs 1	1	8.828	0.04	0.688	0.611
			1 vs 0	1	23.164	0.01	0.853	0.941
	0.3 vs 0.5		5 vs 4	1	0.232	0.66	0.055	0.067
			4 vs 3	1	0.214	0.67	0.051	0.065
			3 vs 2	1	4.876	0.09	0.549	0.394
			2 vs 1	1	0.665	0.46	0.143	0.098
			1 vs 0	1	1.277	0.32	0.242	0.142
	0.5 vs 0.7		5 vs 4	1	3.423	0.14	0.461	0.297
			4 vs 3	1	2.127	0.22	0.347	0.204
			3 vs 2	1	0.150	0.72	0.036	0.061
			2 vs 1	1	6.784	0.06	0.629	0.508
			1 vs 0	1	3.200	0.15	0.444	0.281
	0.7 vs 0.9		5 vs 4	1	1.176	0.34	0.227	0.135
			4 vs 3	1	1.456	0.29	0.267	0.156
			3 vs 2	1	6.689	0.06	0.626	0.502
			2 vs 1	1	1.810	0.25	0.312	0.181
			1 vs 0	1	3.039	0.16	0.432	0.270
	0.9 vs 1.1		5 vs 4	1	0.106	0.76	0.026	0.058
			4 vs 3	1	4.281	0.11	0.517	0.355
			3 vs 2	1	0.603	0.48	0.131	0.093
			2 vs 1	1	0.045	0.84	0.011	0.053
			1 vs 0	1	34.403	<0.01	0.896	0.989
	1.1 vs 1.3		5 vs 4	1	1.383	0.31	0.257	0.150
			4 vs 3	1	0.004	0.96	0.001	0.050
			3 vs 2	1	0.363	0.58	0.083	0.076
			2 vs 1	1	0.254	0.64	0.060	0.068
			1 vs 0	1	0.992	0.38	0.199	0.122
	1.3 vs 1.5		**5 vs 4**	1	8.250	0.05	0.673	0.584
			4 vs 3	1	0.971	0.38	0.195	0.120
			3 vs 2	1	1.402	0.30	0.260	0.152
			2 vs 1	1	0.216	0.67	0.051	0.065
			1 vs 0	1	10.983	0.03	0.733	0.701
		Duration	**X Site**	15	339.037	<0.01	0.988	1.000
		5 vs 10	**5 vs 4**	1	63.328	<0.01	0.941	1.000
			4 vs 3	1	281.557	<0.01	0.986	1.000
			3 vs 2	1	93.196	<0.01	0.959	1.000
			2 vs 1	1	27.912	0.01	0.875	0.970
			1 vs 0	1	38.160	<0.01	0.905	0.993
		10 vs 15	5 vs 4	1	6.706	0.06	0.626	0.503
			4 vs 3	1	12.409	0.02	0.756	0.750
			3 vs 2	1	10.200	0.03	0.718	0.671
			2 vs 1	1	23.423	0.01	0.854	0.944
			1 vs 0	1	662.728	<0.01	0.994	1.000
		15 vs 20	5 vs 4	1	1.640	0.27	0.291	0.169
			4 vs 3	1	0.883	0.40	0.181	0.114
			3 vs 2	1	73.464	<0.01	0.948	1.000
			2 vs 1	1	89.480	<0.01	0.957	1.000
			1 vs 0	1	154.387	<0.01	0.975	1.000

Freq = Frequency; df = degrees of freedom, Eta = Estimated effect size

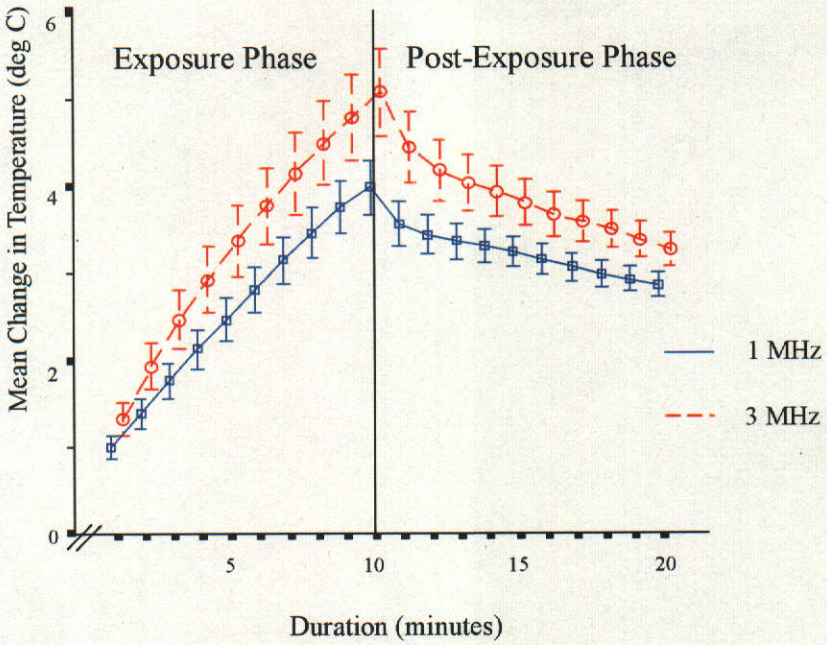

Figure 8.9: Mean (SE) change in temperature for two-way interactions between frequency and duration of exposure (1 to 10 mins) and post-exposure (11 to 20 mins)

(2) Frequency and Duration

There was a significant two-way interaction between frequency and duration of exposure / post-exposure on the change in temperature ($F_{1.321, 5.282}$=9.064, p=0.02, Figure 8.9). A post-hoc analysis (repeated contrasts, within subjects, Table 8.4) demonstrated that there were significant interactions at 1 and 3 MHz:

a. between 10 minutes (exposure) and 15 minutes (post exposure) ($F_{1,4}$=130.888, <0.01);

b. between 15 minutes (post exposure) and 20 minutes (post exposure) ($F_{1,4}$=9.062, p=0.04).

The contrasts showed that the rate of decrease in temperature during the post-exposure phase was greater for 3 MHz than 1 MHz. No other interactions were significant (Table 8.4).

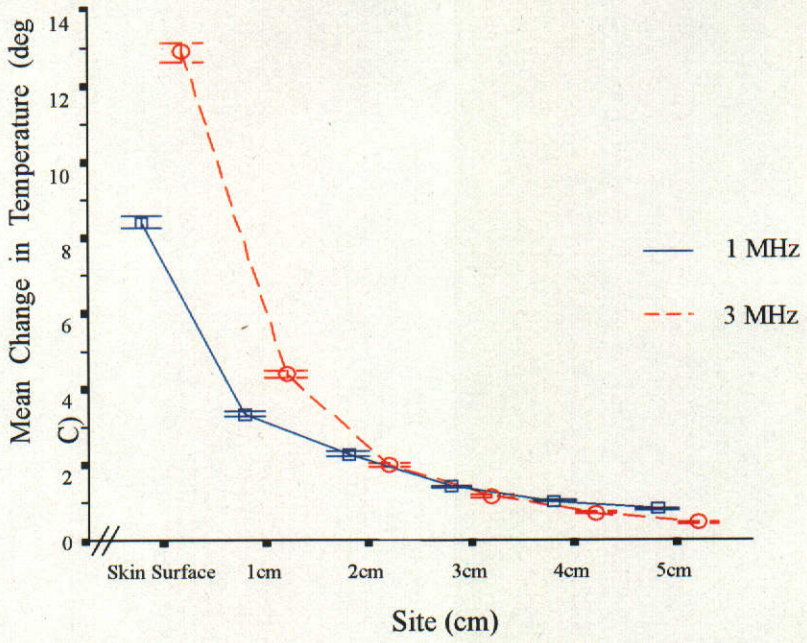

Figure 8.10: Mean (SE) change in temperature for two-way interactions between frequency and site (distance from skin surface)

(3) Frequency and Site

There were significant two-way interactions between frequency and site (distance from skin surface) on the mean change in temperature ($F_{5, 20}$=55.543, $p<0.01$, Figure 8.10). A post-hoc analysis (repeated contrast, within subjects, Table 8.4) demonstrated that there were significant interactions at 1 and 3 MHz:

a. between the skin surface and 1 cm below ($F_{1, 4}$=29.817, $p=0.01$);

b. between 1 cm and 2 cm below ($F_{1, 4}$=47.947, $p<0.01$).

The contrasts showed that the mean change in temperature was greater for the 3 MHz as the distance from the skin surface increased, up to 2 cm below. No other interactions were significant (Table 8.4).

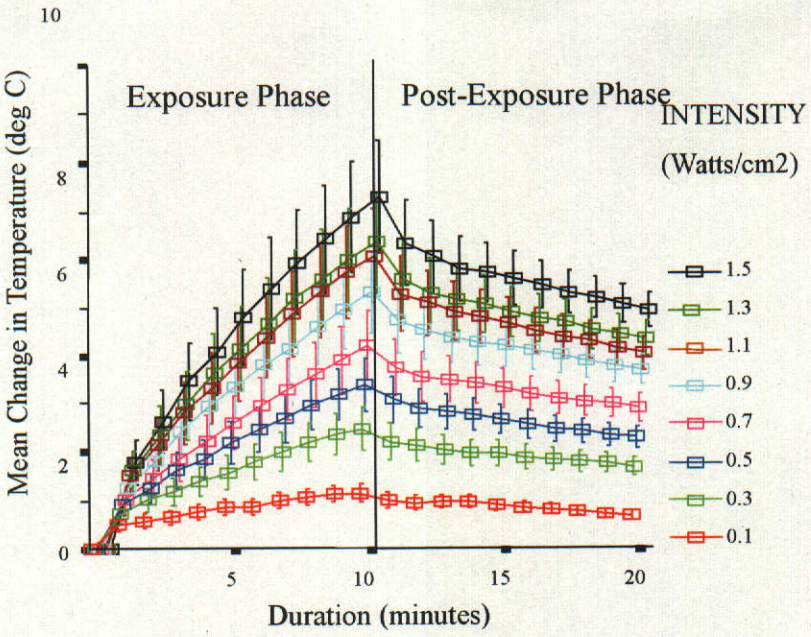

Figure 8.11: Mean (SE) change in temperature for two-way interactions between intensity and duration of exposure (1 to 10 minutes) and post-exposure (11 to 20 minutes)

(4) Intensity and Duration of Exposure

There were significant two-way interactions between intensity and duration of exposure on the mean change in temperature ($F_{21, 84}$=18.657, $p<0.01$, Figures 8.11). A post-hoc analysis (repeated contrast, within subjects, Table 8.4) demonstrated that there were significant interactions:

a. at 5 and 10 minutes

(1) between 0.1 and 0.3 Watts/cm^2 ($F_{1, 4}$=352.200, $p<0.01$);

(2) between 0.3 and 0.5 Watts/cm^2 ($F_{1, 4}$=12.388, $p=0.02$);

(3) between 0.5 and 0.7 Watts/cm^2 ($F_{1, 4}$=18.575, $p=0.01$);

(4) between 0.7 and 0.9 Watts/cm^2 ($F_{1, 4}$=9.908, $p=0.04$);

The contrasts showed that during the exposure phase, the rate of change in temperature increased as the intensity increased from 0.1 to 0.9 Watts/cm^2.

b. at 10 and 15 minutes

(1) between 0.1 compared and 0.3 Watts/cm^2 ($F_{1, 4}$=15.731, p=0.02);

(2) between 0.3 and 0.5 Watts/cm^2 ($F_{1, 4}$=8.593, p=0.04).

The contrasts show that during the initial post-exposure phase (between 10 and 15 minutes), the rate of change in temperature was greatest as the intensity increased from 0.1 to 0.5 Watts/cm^2.

No other interactions were significant (Table 8.4).

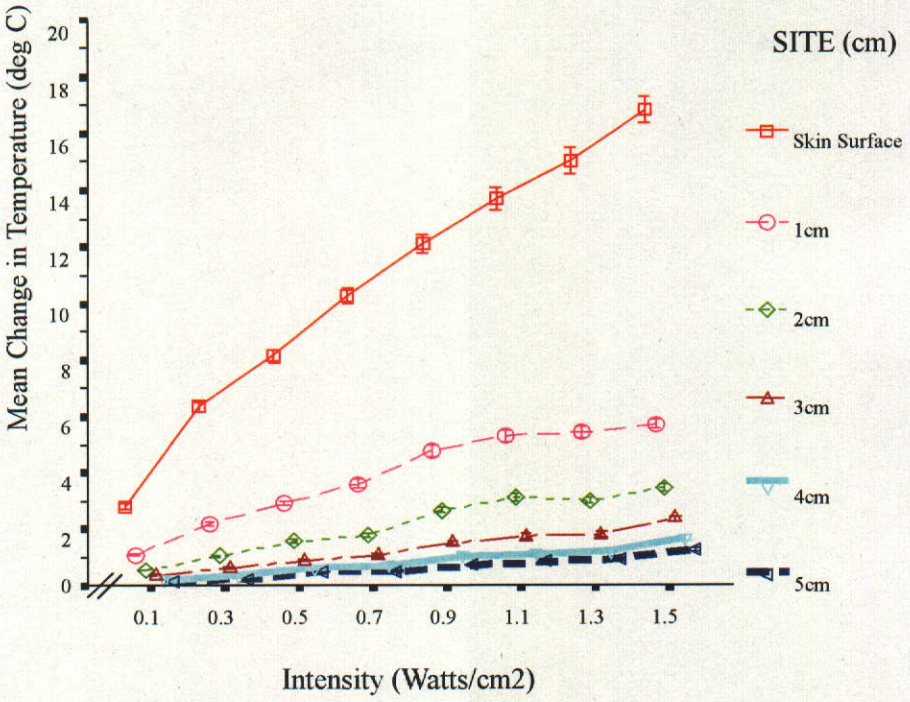

Figure 8.12: Mean (SE) change in temperature for two-way interactions between intensity and site (distance from skin surface)

(5) Intensity and Site

There were significant two-way interactions between intensity and site of target tissue on the change in temperature ($F_{35, 140}$=52.301, $p<0.01$, Figure 8.12). As intensity increased and as distance from the surface decreased, there was a greater and almost linear increase in temperatures. A post-hoc analysis (repeated contrast, within subjects, Table 8.4) demonstrated that there were significant interactions:

a. at 0.1 and 0.3 Watts/cm^2:

(1) between 3 and 2 cm ($F_{1, 4}$=10.686, p=0.03);

(2) between 2 and 1 cm ($F_{1, 4}$=8.828, p=0.04);

(3) between 1 cm and skin surface ($F_{1, 4}$=23.164, p=0.01);

The contrasts showed that the greatest change in temperature occurred at the skin surface and up to 3 cm below the skin surface

as intensity increased from 0.1 to 0.3 Watts/cm^2.

b. at 0.9 and 1.1 Watts/cm^2:

(1) between 1 cm and skin surface ($F_{1,4}$=34.403, $p<0.01$);

The contrasts showed that the greatest change in temperature occurred at the skin surface and up to 1 cm below the skin surface as intensity increased from 0.9 to 1.1 Watts/cm^2.

c. at 1.3 and 1.5 Watts/cm^2:

(1) between 5 and 4 cm ($F_{1,4}$=8.250, $p=0.05$)

(2) between 1 cm and skin surface ($F_{1,4}$=10.983, $p<0.03$)

The contrasts showed that the greatest change in temperature occurred at the skin surface and up to 1 cm below, and from 4 to 5 cm below, as intensity increased from 1.3 to 1.5 Watts/cm^2.

Collectively, the contrasts showed that the significant difference in the change in temperatures was confined to the lowest and highest intensities, and these were confined mainly to the superficial tissues.

No other interactions were significant (Table 8.4).

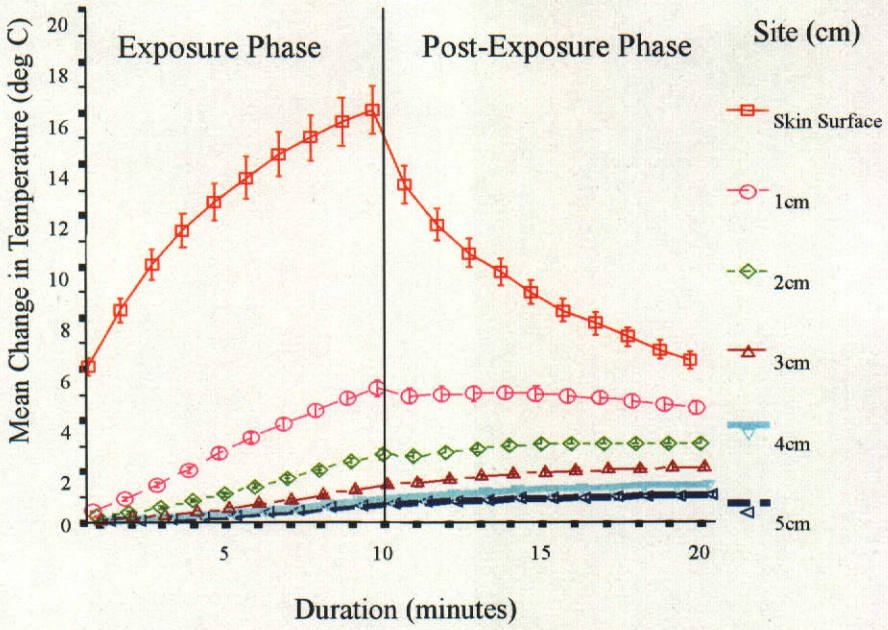

Figure 8.13: Mean (SE) change in temperature for two-way interactions between site and duration of exposure (1 to 10 minutes) and post-exposure (11 to 20 minutes)

(6) Duration and Site

There were significant two-way interactions between duration of exposure and site of target tissue on the change in temperature ($F_{15, 60}$=339.037, p<0.01, Figures 8.13). A post-hoc analysis (repeated contrasts, within subjects, Table 8.4) demonstrated that there were significant interactions:

a. at 5 and 10 minutes

(1) between skin surface and 1 cm ($F_{1, 4}$=38.160, p<0.01);

(2) between 1 cm and 2 cm ($F_{1, 4}$=27.912, p=0.01);

(3) between 2 cm and 3 cm ($F_{1, 4}$=93.196, p<0.01);

(4) between 3 cm and 4 cm ($F_{1, 4}$=281.557, p<0.01);

(5) between 4 cm and 5 cm ($F_{1, 4}$=63.328, p<0.01);

The contrasts showed that as the duration of exposure increased from 5 to 10 minutes, the rate of change in temperature was greater as the distance from the skin surface decreased from 5 cm to skin

surface.

b. at 10 and 15 minutes

(1) between skin surface and 1 cm ($F_{1,4}$=662.728, $p<0.01$);

(2) between 1 cm and 2 cm ($F_{1,4}$=23.423, $p=0.01$);

(3) between 2 cm and 3 cm ($F_{1,4}$=10.200, $p=0.03$);

(4) between 3 cm and 4 cm ($F_{1,4}$=12.409, $p=0.02$);

The contrasts showed that as the duration of post-exposure increased from 10 to 15 minutes, the rate of change in temperature was greater as the distance from the skin surface decreased from 4 cm to skin surface. In particular, the difference in the rate of decrease in temperature between the skin surface and 1 cm below was most noticeable. For distances between 2 and 4 cm below the surface, the rate of increase in temperature was greater as the distance from the surface decreased.

c. at 15 and 20 minutes

(1) between skin surface and 1 cm ($F_{1,4}$=154.387, $p<0.01$);

(2) between 1 cm and 2 cm ($F_{1,4}$=89.480, $p<0.01$);

(3) between 2 cm and 3 cm ($F_{1,4}$=73.464, $p<0.01$).

The contrasts showed that as the duration of post-exposure increased from 15 to 20 minutes, the rate of change in temperature was similar, although less noticeable, to that described for 10 to 15 minutes.

Together, the contrasts indicated that as the duration of exposure and post-exposure increased, the significant interactions at the various tissue depths occurred more superficially. No other interactions were significant (Table 8.4).

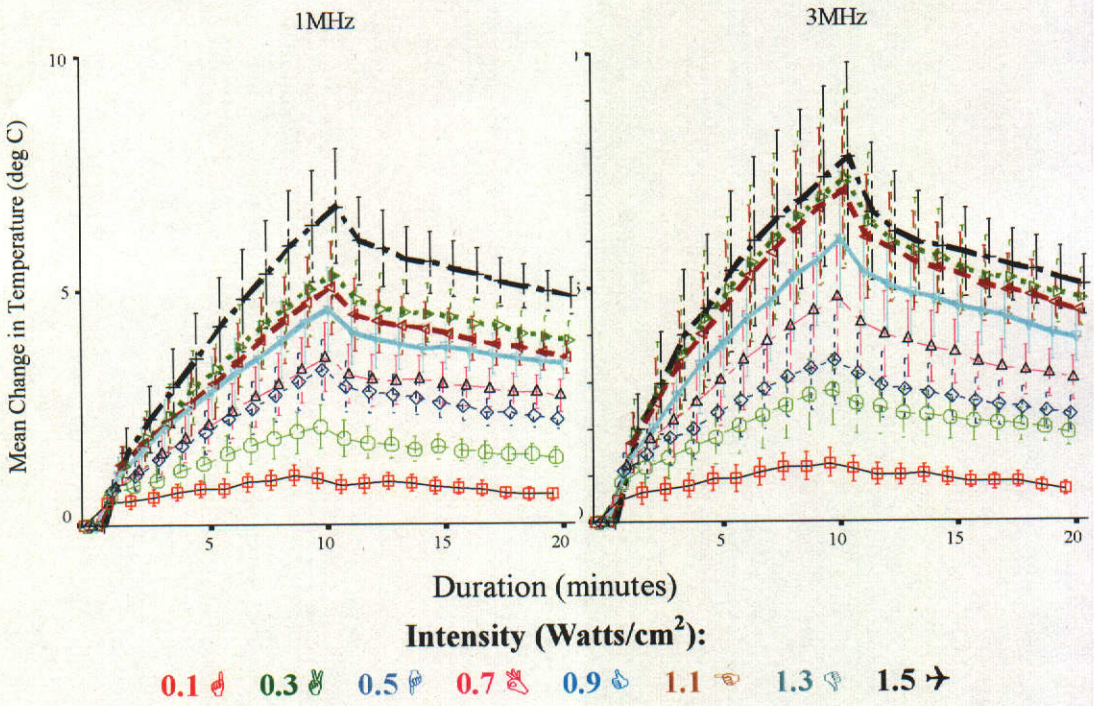

Figure 8.14: Mean (SE) change in temperature for three-way interactions among frequency, intensity and duration

d. Results of analysis for three-way interactions

(1) Frequency, Intensity and Duration of Exposure

There were significant three-way interactions among frequency, intensity and duration of exposure on the temperature increase ($F_{21, 84}$=2.612, $p<0.01$, Figure 8.14). As intensity and duration of exposure / post-exposure increased, the rate of change in temperature was greater for 3 MHz compared with 1 MHz. A post-hoc analysis (repeated contrasts, within subjects, Table 8.5) demonstrated that there were significant interactions:

a. at 1 and 3 MHz, and at 5 and 10 minutes

(1) between 0.1 and 0.3 Watts/cm^2 ($F_{1, 4}$=8.996, p=0.04);

(2) between 0.3 and 0.5 Watts/cm^2 ($F_{1, 4}$=11.991, p=0.03);

(3) between 0.5 and 0.7 Watts/cm^2 ($F_{1, 4}$=15.477, p=0.02);

The contrasts showed that as the duration of exposure increased

from 5 to 10 minutes, and as the intensity increased from 0.1 to 0.7 Watts/cm^2, the rate of change in temperature was greater for 3 MHz.

b. at 1 and 3 MHz, and at 10 and 15 minutes

(1) between 0.5 and 0.7 Watts/cm^2 ($F_{1,4}$=14.632, p=0.02).

The contrasts showed that as the duration of exposure increased from 10 to 15 minutes, and as the intensity increased from 0.5 to 0.7 Watts/cm^2, the rate of change in temperature was greater for 3 MHz.

Collectively, the contrasts showed that as duration increased, the rate of change in temperature was greater for 3 MHz, and this was confined mainly to the lower intensities up to 0.7 Watts/cm^2.

No other interactions were significant (Table 8.5).

Table 8.5: Repeated Measures ANOVA (General Linear Model, within subjects effects), and Contrasts (within subjects, repeated) for the three-way interaction effects on main factors (Frequency, Intensity, Duration, Site) (Bold fonts indicate significant interactions)

Factor				df	F value	p value	Eta	Power
Freq X	**Intensity X**	**Duration**		21	2.612	<0.01	0.395	0.996
1 vs 3	**0.1 vs 0.3**	**5 vs 10**		1	8.996	0.04	0.692	0.619
	0.3 vs 0.5			1	11.991	0.03	0.750	0.736
	0.5 vs 0.7			1	15.477	0.02	0.795	0.832
	0. 7vs 0.9			1	0.152	0.72	0.037	0.061
	0.9 vs 1.1			1	0.015	0.91	0.004	0.051
	1.1 vs 1.3			1	0.010	0.92	0.003	0.051
	1.3 vs 1.5			1	5.011	0.09	0.556	0.402
	0.1 vs 0.3	**10 vs 15**		1	0.115	0.75	0.028	0.058
	0.3 vs 0.5			1	0.127	0.74	0.031	0.059
	0.5 vs 0.7			1	14.632	0.02	0.785	0.812
	0. 7vs 0.9			1	0.001	0.98	0.000	0.050
	0.9 vs 1.1			1	1.536	0.28	0.278	0.161
	1.1 vs 1.3			1	0.975	0.38	0.196	0.120
	1.3 vs 1.5			1	0.963	0.38	0.194	0.120
	0.1 vs 0.3	15 vs 20		1	0.004	0.95	0.001	0.050
	0.3 vs 0.5			1	0.884	0.40	0.181	0.114
	0.5 vs 0.7			1	4.474	0.10	0.528	0.367
	0. 7vs 0.9			1	0.000	1.00	0.000	0.050
	0.9 vs 1.1			1	0.551	0.50	0.121	0.090
	1.1 vs 1.3			1	0.443	0.54	0.100	0.082
	1.3 vs 1.5			1	0.040	0.85	0.010	0.053
Freq X	**Intensity**	**X**	**Site**	35	5.341	<0.01	0.572	1.000
1 vs 3	0.1 vs 0.3		5 vs 4	1	0.265	0.63	0.062	0.069
			4 vs 3	1	3.629	0.13	0.476	0.311
			3 vs 2	1	0.005	0.95	0.001	0.050
			2 vs 1	1	2.347	0.20	0.370	0.220
			1 vs 0	1	2.753	0.17	0.408	0.249
	0.3 vs 0.5		5 vs 4	1	0.105	0.76	0.025	0.057
			4 vs 3	1	1.982	0.23	0.331	0.194
			3 vs 2	1	2.185	0.21	0.353	0.209
			2 vs 1	1	0.525	0.51	0.116	0.088
			1 vs 0	1	0.227	0.66	0.054	0.066
	0.5 vs 0.7		5 vs 4	1	4.355	0.11	0.521	0.360
			4 vs 3	1	3.490	0.14	0.466	0.301
			3 vs 2	1	2.017	0.23	0.335	0.197
			2 vs 1	1	0.638	0.47	0.138	0.096
			1 vs 0	1	0.119	0.75	0.029	0.058
	0.7 vs 0.9		5 vs 4	1	0.063	0.81	0.015	0.054
			4 vs 3	1	0.254	0.64	0.060	0.068
			3 vs 2	1	6.461	0.06	0.618	0.489
			2 vs 1	1	0.066	0.81	0.016	0.055
			1 vs 0	1	0.089	0.78	0.022	0.056
	0.9 vs 1.1		5 vs 4	1	0.702	0.45	0.149	0.101
			4 vs 3	1	0.891	0.40	0.182	0.114
			3 vs 2	1	8.466	0.04	0.679	0.594
			2 vs 1	1	8.703	0.04	0.685	0.606
			1 vs 0	1	2.307	0.20	0.366	0.217

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Table 8.5: continued from previous page

Factor				df	F value	p value	Eta	Power
Freq X	Intensity X	DurationX	Site					
	1.1 vs 1.3		5 vs 4	1	0.506	0.52	0.112	0.086
			4 vs 3	1	7.047	0.06	0.638	0.522
			3 vs 2	1	0.001	0.98	0.000	0.050
			2 vs 1	1	0.416	0.55	0.094	0.080
			1 vs 0	1	0.908	0.40	0.185	0.116
	1.3 vs 1.5		5 vs 4	1	1.750	0.26	0.304	0.177
			4 vs 3	1	4.573	0.10	0.533	0.374
			3 vs 2	1	0.010	0.92	0.003	0.051
			2 vs 1	1	0.518	0.51	0.115	0.087
			1 vs 0	1	0.014	0.91	0.003	0.051
	Intensity X	**Duration**	**X Site**	105	42.523	<0.01	0.914	1.000
	0.1 vs 0.3	**5 vs 10**	5 vs 4	1	0.016	0.91	0.004	0.051
			4 vs 3	1	2.139	0.22	0.348	0.205
			3 vs 2	1	34.803	<0.01	0.897	0.989
			2 vs 1	1	20.441	0.01	0.836	0.914
			1 vs 0	1	14.265	0.02	0.781	0.803
	0.3 vs 0.5		5 vs 4	1	1.067	0.36	0.211	0.127
			4 vs 3	1	0.865	0.41	0.178	0.112
			3 vs 2	1	0.274	0.63	0.064	0.070
			2 vs 1	1	15.091	0.02	0.790	0.823
			1 vs 0	1	0.411	0.56	0.093	0.079
	0.5 vs 0.7		5 vs 4	1	0.138	0.73	0.033	0.060
			4 vs 3	1	4.000	0.12	0.500	0.336
			3 vs 2	1	0.026	0.88	0.006	0.052
			2 vs 1	1	0.258	0.64	0.061	0.068
			1 vs 0	1	7.952	0.05	0.665	0.569
	0.7 vs 0.9		5 vs 4	1	0.008	0.93	0.002	0.051
			4 vs 3	1	0.364	0.58	0.083	0.076
			3 vs 2	1	10.629	0.03	0.727	0.688
			2 vs 1	1	0.348	0.58	0.080	0.075
			1 vs 0	1	0.671	0.46	0.144	0.098
	0.9 vs 1.1		5 vs 4	1	0.783	0.43	0.164	0.106
			4 vs 3	1	0.031	0.87	0.008	0.052
			3 vs 2	1	0.166	0.71	0.040	0.062
			2 vs 1	1	2.281	0.21	0.363	0.216
			1 vs 0	1	0.064	0.81	0.016	0.055
	1.1 vs 1.3		5 vs 4	1	0.339	0.59	0.078	0.074
			4 vs 3	1	0.699	0.45	0.149	0.100
			3 vs 2	1	0.028	0.88	0.007	0.052
			2 vs 1	1	0.418	0.55	0.095	0.080
			1 vs 0	1	0.246	0.65	0.058	0.068
	1.3 vs 1.5		5 vs 4	1	0.252	0.64	0.059	0.068
			4 vs 3	1	0.650	0.47	0.140	0.097
			3 vs 2	1	1.237	0.33	0.236	0.140
			2 vs 1	1	0.517	0.51	0.114	0.087
			1 vs 0	1	1.361	0.31	0.254	0.149
	0.1 vs 0.3	**10 vs 15**	5 vs 4	1	1.361	0.31	0.254	0.149
			4 vs 3	1	8.818	0.04	0.688	0.611
			3 vs 2	1	0.025	0.88	0.006	0.052
			2 vs 1	1	1.593	0.28	0.285	0.166
			1 vs 0	1	13.822	0.02	0.776	0.791

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Table 8.5: continued from previous page

Factor				df	F value	p value	Eta	Power
Freq X	Intensity X	DurationX	Site					
	0.3 vs 0.5		5 vs 4	**1**	0.513	0.51	0.114	0.087
			4 vs 3	**1**	0.022	0.89	0.006	0.052
			3 vs 2	**1**	0.140	0.73	0.034	0.060
			2 vs 1	**1**	0.432	0.55	0.097	0.081
			1 vs 0	1	9.297	0.04	0.699	0.633
	0.5 vs 0.7		5 vs 4	1	2.934	0.16	0.423	0.262
			4 vs 3	1	0.810	0.42	0.168	0.108
			3 vs 2	1	0.747	0.44	0.157	0.104
			2 vs 1	1	0.114	0.75	0.028	0.058
			1 vs 0	1	7.084	0.06	0.639	0.524
	0.7 vs 0.9		5 vs 4	1	0.656	0.46	0.141	0.097
			4 vs 3	1	0.591	0.49	0.129	0.092
			3 vs 2	1	0.168	0.70	0.040	0.062
			2 vs 1	1	0.150	0.72	0.036	0.061
			1 vs 0	1	11.826	0.03	0.747	0.731
	0.9 vs 1.1		5 vs 4	1	2.863	0.17	0.417	0.257
			4 vs 3	1	0.573	0.49	0.125	0.091
			3 vs 2	1	0.460	0.54	0.103	0.083
			2 vs 1	1	2.236	0.21	0.359	0.212
			1 vs 0	1	14.805	0.02	0.787	0.817
	1.1 vs 1.3		5 vs 4	1	0.719	0.44	0.152	0.102
			4 vs 3	1	0.356	0.58	0.082	0.076
			3 vs 2	1	0.254	0.64	0.060	0.068
			2 vs 1	1	0.027	0.88	0.007	0.052
			1 vs 0	1	2.809	0.17	0.413	0.253
	1.3 vs 1.5		5 vs 4	1	0.082	0.79	0.020	0.056
			4 vs 3	1	0.096	0.77	0.024	0.057
			3 vs 2	1	74.699	<0.01	0.949	1.000
			2 vs 1	1	2.199	0.21	0.355	0.210
			1 vs 0	1	3.350	0.14	0.456	0.291
	0.1 vs 0.3	15 vs 20	5 vs 4	1	1.887	0.24	0.321	0.187
			4 vs 3	1	0.117	0.75	0.028	0.058
			3 vs 2	1	0.010	0.92	0.003	0.051
			2 vs 1	1	2.291	0.21	0.364	0.216
			1 vs 0	1	3.748	0.13	0.484	0.319
	0.3 vs 0.5		5 vs 4	1	0.757	0.43	0.159	0.105
			4 vs 3	1	3.356	0.14	0.456	0.292
			3 vs 2	1	2.049	0.23	0.339	0.199
			2 vs 1	1	0.760	0.43	0.160	0.105
			1 vs 0	1	2.599	0.18	0.394	0.238
	0.5 vs 0.7		5 vs 4	1	0.175	0.70	0.042	0.062
			4 vs 3	1	18.856	0.01	0.825	0.893
			3 vs 2	1	3.851	0.12	0.491	0.326
			2 vs 1	1	0.011	0.92	0.003	0.051
			1 vs 0	1	1.069	0.36	0.211	0.127
	0.7 vs 0.9		5 vs 4	1	4.195	0.11	0.512	0.349
			4 vs 3	1	2.258	0.21	0.361	0.214
			3 vs 2	1	37.647	<0.01	0.904	0.993
			2 vs 1	1	0.643	0.47	0.138	0.096
			1 vs 0	1	0.086	0.78	0.021	0.056

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Table 8.5: continued from previous page

Factor				df	F value	p value	Eta	Power
Freq X	Intensity X	DurationX	Site					
	0.9 vs 1.1		**5 vs 4**	1	11.901	0.03	0.748	0.733
			4 vs 3	1	5.859	0.07	0.594	0.454
			3 vs 2	1	5.538	0.08	0.581	0.435
			2 vs 1	1	8.846	0.04	0.689	0.612
			1 vs 0	1	1.971	0.23	0.330	0.193
	1.1 vs 1.3		5 vs 4	1	2.998	0.16	0.428	0.267
			4 vs 3	1	0.123	0.74	0.030	0.059
			3 vs 2	1	0.014	0.91	0.004	0.051
			2 vs 1	1	0.173	0.70	0.041	0.062
			1 vs 0	1	0.152	0.72	0.037	0.061
	1.3 vs 1.5		5 vs 4	1	2.834	0.17	0.415	0.255
			4 vs 3	1	1.952	0.24	0.328	0.192
			3 vs 2	1	0.023	0.89	0.006	0.052
			2 vs 1	1	0.384	0.57	0.088	0.077
			1 vs 0	1	0.775	0.43	0.162	0.106
Freq	**X**	**Duration**	**X Site**	15	51.494	<0.01	0.928	1.000
1 vs 3		**5 vs 10**	5 vs 4	1	0.012	0.92	0.003	0.051
			4 vs 3	1	0.750	0.44	0.158	0.104
			3 vs 2	1	.0.947	0.39	0.191	0.118
			2 vs 1	1	39.456	<0.01	0.908	0.995
			1 vs 0	1	4.354	0.11	0.521	0.360
		10 vs 15	5 vs 4	1	3.121	0.15	0.438	0.275
			4 vs 3	1	7.176	0.06	0.642	0.529
			3 vs 2	1	4.511	0.10	0.530	0.370
			2 vs 1	1	6.013	0.07	0.601	0.463
			1 vs 0	1	33.664	<0.01	0.894	0.987
		15 vs 20	5 vs 4	1	0.072	0.80	0.018	0.055
			4 vs 3	1	4.554	0.10	0.532	0.373
			3 vs 2	1	0.564	0.49	0.124	0.091
			2 vs 1	1	3.045	0.16	0.432	0.270
			1 vs 0	1	72.153	<0.01	0.947	1.000

Freq = Frequency; df = degrees of freedom, Eta = Estimated effect size

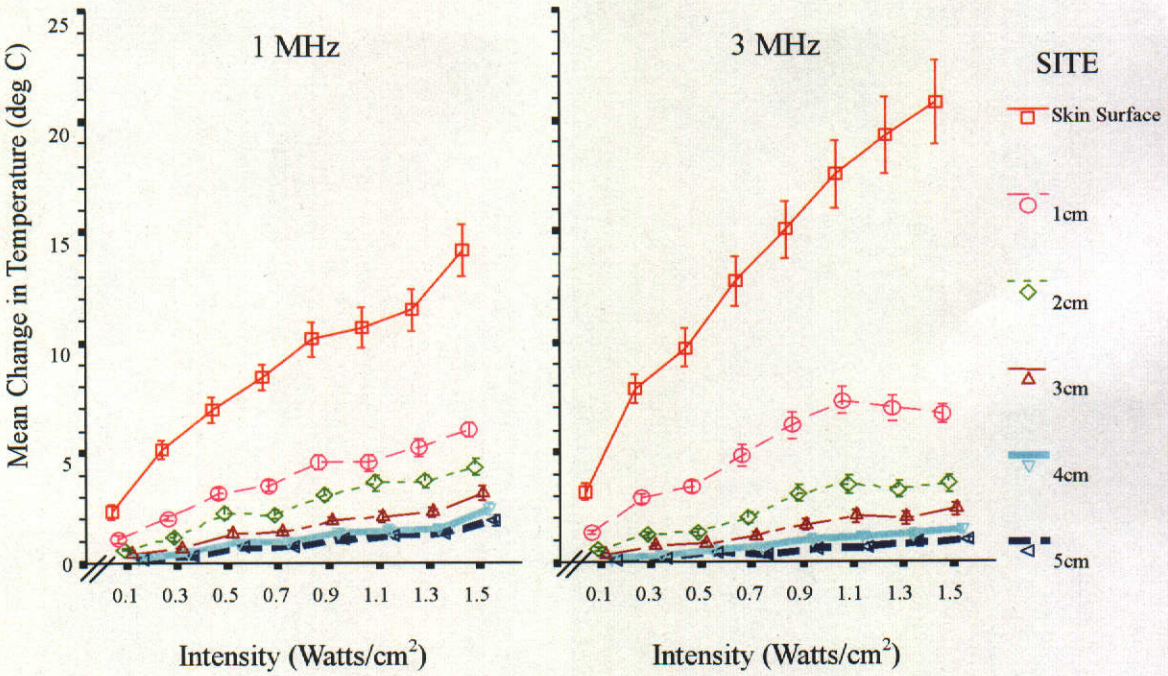

Figure 8.15: Mean (SE) change in temperature for three-way interactions among frequency, intensity and site (distance from skin surface)

(2) Frequency, Intensity and Site

There were significant three-way interactions among frequency, intensity and site on the change in temperature ($F_{35, 140}$=5.341, $p<0.01$, Figure 8.15). A post-hoc analysis (repeated contrasts, within subjects, Table 8.5) demonstrated that there were significant interactions:

a. at 1 and 3 MHz and at 0.9 and 1.1 Watts/cm^2

(1) between 1 cm and 2 cm ($F_{1, 4}$=8.703, p=0.04);

(2) between 2 cm and 3 cm ($F_{1, 4}$=8.466, p=0.04).

The contrasts showed that the change in temperature was greater for the 3MHz frequency as the intensity increased from 0.9 to 1.1 Watts/cm^2 and as the distance from the surface decreased from 3 cm to 1 cm.

No other interactions were significant (Table 8.5).

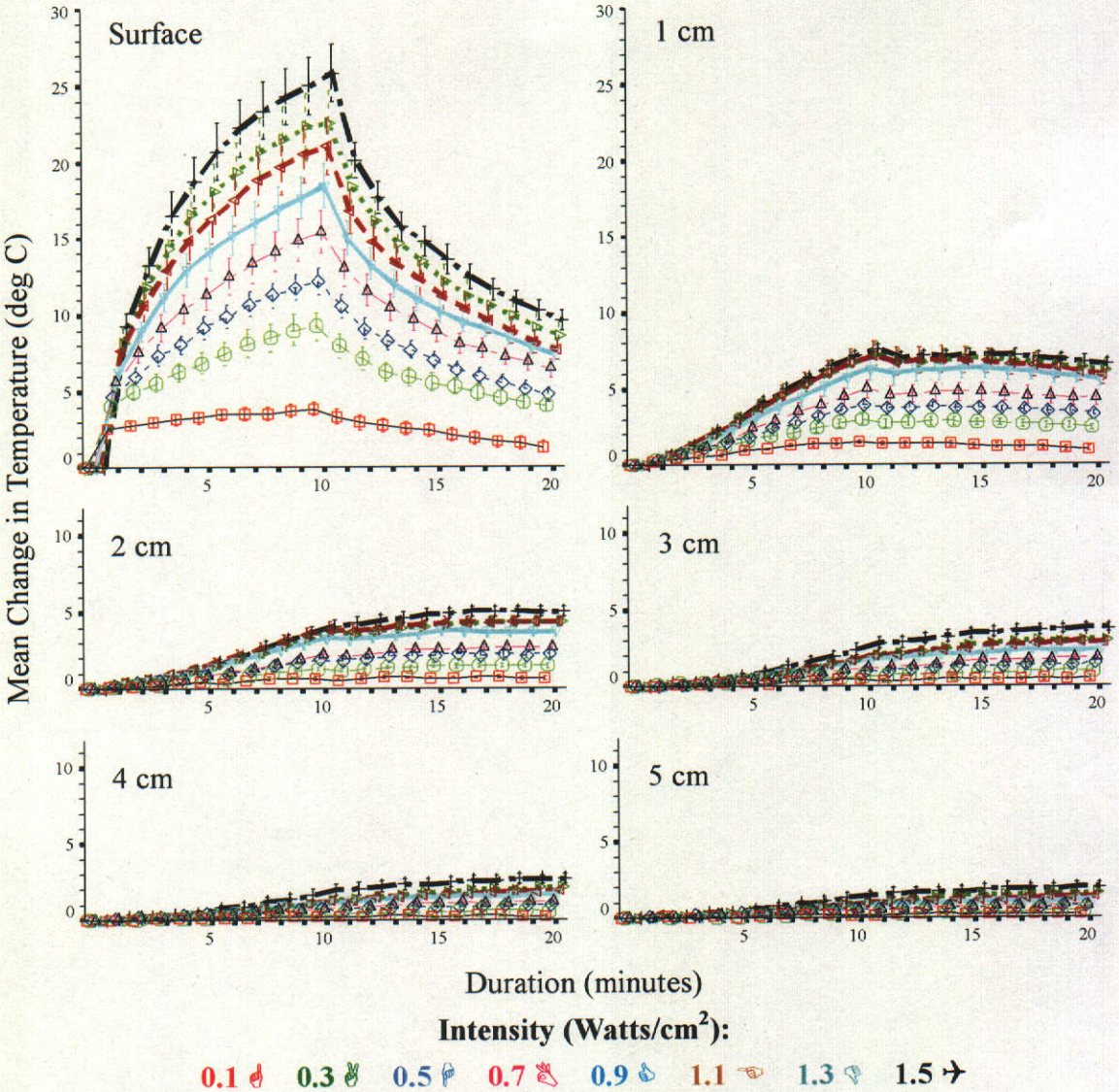

Figure 8.16: Mean (SE) change in temperature for three-way interactions among intensity, site (distance from skin surface) and duration of exposure (5 and 10 mins) and post-exposure (15 and 20 mins).

(3) Intensity, Duration of Exposure and Site

There were significant three-way interactions among intensity, duration of exposure and site on the rate of change in temperature ($F_{105, 420}$=42.523, $p<0.01$, Figure 8.16). A post-hoc analysis (repeated contrasts, within subjects, Table 8.5) demonstrated that there were significant interactions:

a. at 5 and 10 minutes, and at 0.1 and 0.3 Watts/cm^2

(1) between skin surface and 1 cm ($F_{1,4}$=14.265, p=0.02);

(2) between 1 cm and 2 cm ($F_{1,4}$=20.441, p=0.01);

(3) between 2 cm and 3 cm ($F_{1,4}$=34.803, p<0.01);

b. at 5 and 10 minutes, and at 0.3 and 0.5 Watts/cm^2

(1) between 1 cm and 2 cm ($F_{1,4}$=15.091, p=0.02);

c. at 5 and 10 minutes, and at 0.5 and 0.7 Watts/cm^2

(1) between skin surface and 1 cm ($F_{1,4}$=7.952, p=0.05);

d. at 5 and 10 minutes, and at 0.7 and 0.9 Watts/cm^2

(1) between 2 cm and 3 cm ($F_{1,4}$=10.629, p=0.03);

These contrasts showed that as the duration of exposure increased from 5 to 10 minutes, the rate of change in temperatures was significantly greater as intensities increased from 0.1 to 0.9 Watts/cm^2, and was confined mainly to the superficial tissues (less than 3 cm below surface).

e. at 10 and 15 minutes, and at 0.1 and 0.3 Watts/cm^2

(1) between skin surface and 1 cm ($F_{1,4}$=13.822, p=0.02);

(2) between 3 cm and 4 cm ($F_{1,4}$=8.818, p=0.04);

f. at 10 and 15 minutes, and at 0.3 and 0.5 Watts/cm^2

(1) between skin surface and 1 cm ($F_{1,4}$=9.297, p=0.04);

g. at 10 and 15 minutes, and at 0.7 and 0.9 Watts/cm^2

(1) between skin surface and 1 cm ($F_{1,4}$=11.826, p=0.03);

i. at 10 and 15 minutes, and at 0.9 and 1.1 Watts/cm^2

(1) between skin surface and 1 cm ($F_{1,4}$=14.805, p=0.02);

j. at 10 and 15 minutes, and at 1.3 and 1.5 Watts/cm^2

(1) between 2 cm and 3 cm ($F_{1,4}$=74.699, p<0.01);

These contrasts showed that as the duration of post-exposure increased from 10 to 15 minutes, the rate of change in temperatures was significantly

greater as intensities increased from 0.1 to 1.5 Watts/cm^2 (except for between 0.5 and 0.7, and 1.1 and 1.3 Watts/cm^2) and was confined mainly to the superficial tissues (less than 4 cm below surface).

k. at 15 and 20 minutes at 0.5 and 0.7 Watts/cm^2

(1) between 3 cm and 4 cm ($F_{1, 4}$=18.856, p=0.01);

l. at 15 and 20 minutes and at 0.7 and 0.9 Watts/cm^2

(1) between 2 cm and 3 cm ($F_{1, 4}$=37.647, p<0.01);

m. at 15 and 20 minutes, and at 0.9 and 1.1 Watts/cm^2

(1) between 1 cm and 2 cm ($F_{1, 4}$=8.846, p=0.04);

(2) between 4 cm and 5 cm ($F_{1, 4}$=11.901, p=0.03)

These contrasts showed that as the duration of post-exposure increased from 15 to 20 minutes, the rate of change in temperatures was significantly greater as intensities increased from 0.5 to 1.1 Watts/cm^2, and was confined mainly to the subcutaneous tissues (1 to 5 cm below surface).

Collectively, these contrasts show that during the exposure phase (between 5 and 10 minutes), the significant interactions were confined to the superficial tissues (from skin surface to 3 cm below) between each adjacent intensity ranging from 0.1 to 0.9 Watts/cm^2. During the initial post-exposure phase (between 10 and 15 minutes), the significant interactions were confined to the middle portion of the tissues (from skin surface to 4 cm below) between each adjacent intensity ranging from 0.1 to 1.5 Watts/cm^2 (except for between 0.5 and 0.7, and 1.1 and 1.3 Watts/cm^2). During the late post-exposure phase (between 15 and 20 minutes), the significant interactions were confined mainly to the deeper tissues (from 1 to 5 cm) between each adjacent intensity ranging from 0.5 to 1.1 Watts/cm^2. No other interactions were significant (Table 8.5).

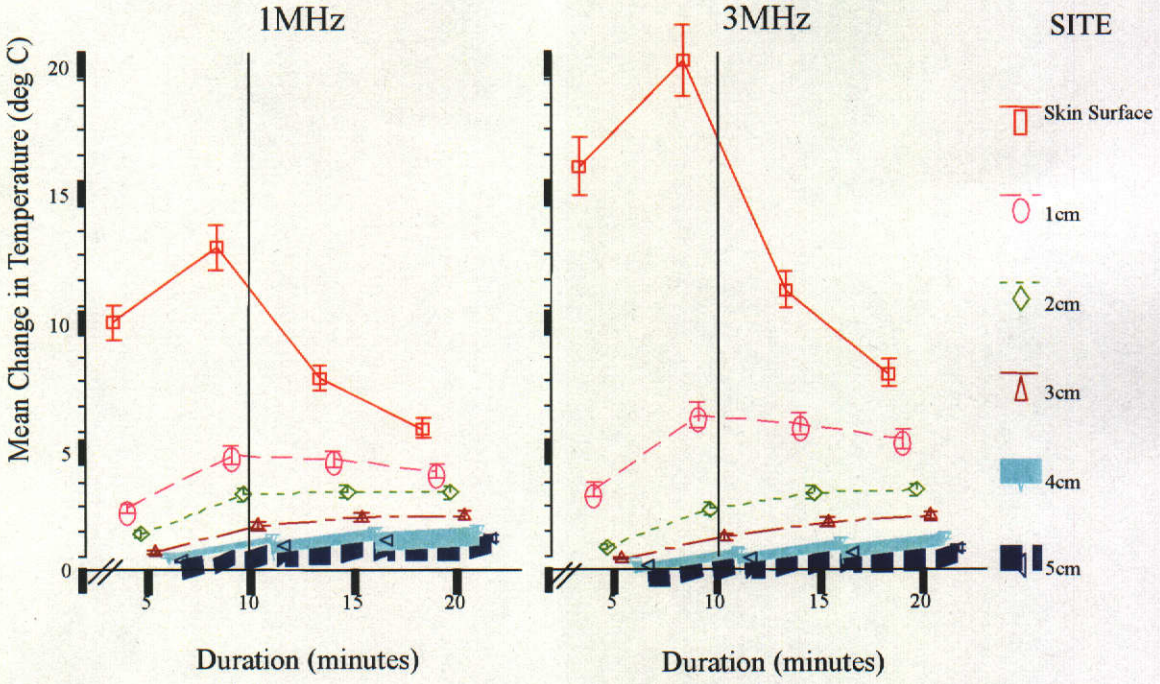

Figure 8.17: Mean (SE) change in temperature for three-way interactions among frequency, site (distance from skin surface) and duration of exposure (5 and 10 mins) and post-exposure (15 and 20 mins).

(4) Frequency, Duration of Exposure and Site

There were significant three-way interactions among frequency, duration of exposure and site on the rate of change in temperature ($F_{15, 60}$=51.494, p<0.01, Figure 8.17). A post-hoc analysis (repeated contrasts, within subjects, Table 8.5) demonstrated that there were significant interactions:

a. at 1 and 3 MHz, and at 5 and 10 minutes,

(1) between 1 cm and 2 cm ($F_{1, 4}$=39.456, p<0.01);

b. at 1 and 3 MHz, and at 10 and 15 minutes,

(1) between skin surface and 1 cm ($F_{1, 4}$=33.664, p<0.01);

c. at 1 and 3 MHz, and at 15 and 20 minutes,

(1) between skin surface and 1 cm ($F_{1, 4}$=72.153, p<0.01).

The contrasts showed that between 1 and 3 MHz during the late post-exposure phase, the significant interactions occurred between adjacent tissue sites from 1 to 2 cm.

Collectively, the contrasts showed that as the duration of exposure and post-exposure increased, the rate of change in temperatures was greater for the 3 MHz frequency, and these were confined to the superficial tissues (less than 2 cm below surface). No other interactions were significant (Table 8.5).

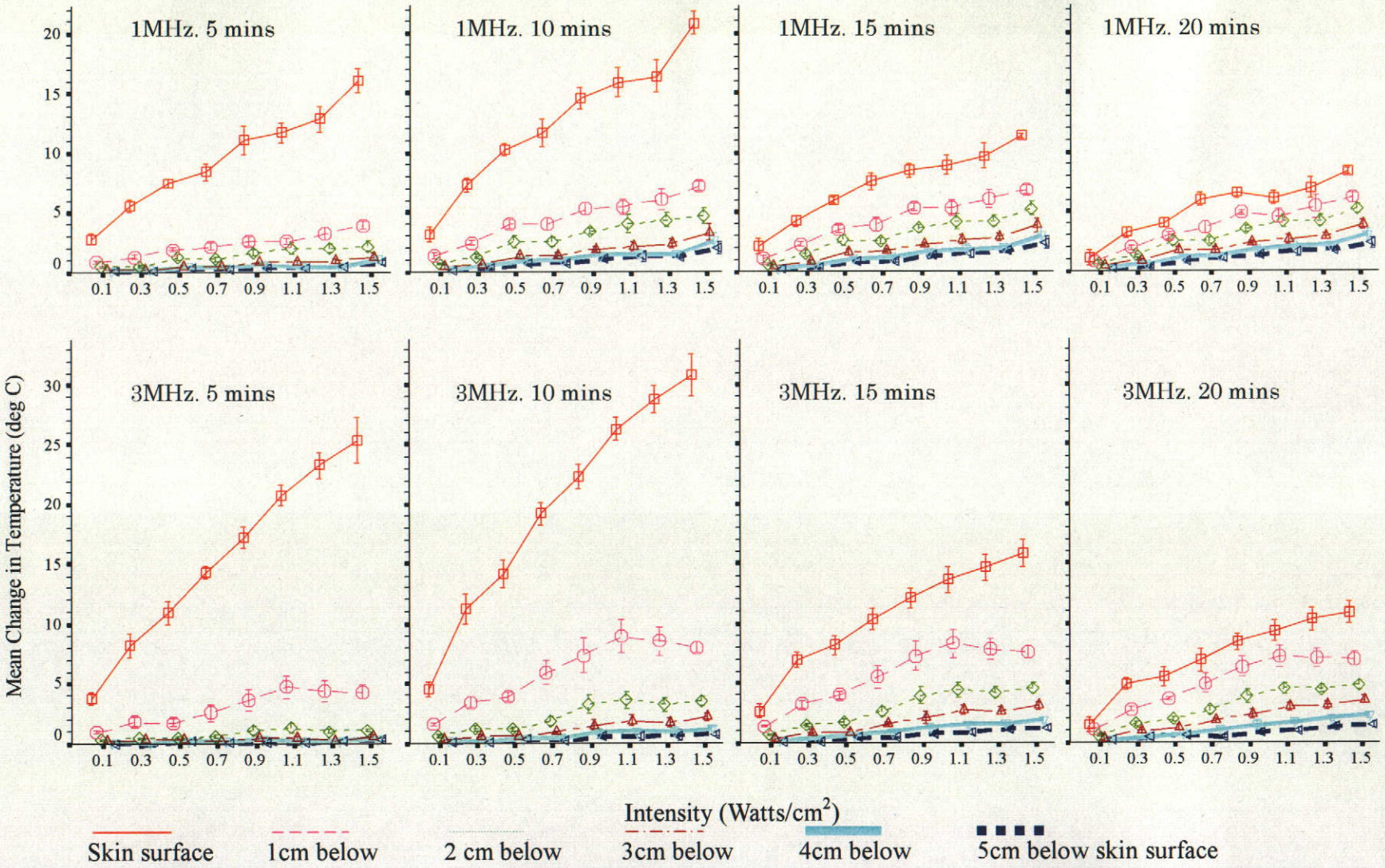

Figure 8.18: Mean (1SE) change in temperature for four-way interactions among frequency, intensity, site (distance from skin surface) and duration of exposure (5 and 10 mins) and post-exposure (15 and 20 mins).

e. Results of analysis for four-way interactions

(1) Frequency, Intensity, Duration of Exposure and Site

Figure 8.18 summarises the four way interactions among frequency, intensity, duration of exposure and site of target tissue, with respect to frequency and duration. In the "General" results section, Figures 8.2 and 8.3 also summarise the four-way interactions, with respect to frequency and site, for 1 and 3 MHz respectively. In order to facilitate understanding of the four-way interactions, it may be necessary to refer to these three figures. There were significant four-way interactions among frequency, intensity, duration of exposure and site on the change in temperature ($F_{105, 420}$=5.875, $p<0.01$, Figure 8.18). A post-hoc analysis (repeated contrasts, within subjects, Table 8.6) demonstrated that there were significant interactions:

a. at 1 and 3 MHz, at 5 and 10 minutes and at 0.5 and 0.7 Watts/cm^2,

(1) between 1 cm and 2 cm ($F_{1, 4}$=33.726, $p<0.01$);

The contrast showed that as the duration of exposure increased from 5 to 10 minutes, the rate of change in temperature was greater for the 3 MHz compared with the 1 MHz between 0.5 and 0.7 Watts/cm2.

b. at 1 and 3 MHz, at 10 and 15 minutes and at 0.1 and 0.3 Watts/cm^2,

(1) between 2 cm and 3 cm ($F_{1, 4}$=38.252, $p<0.01$);

c. at 1 and 3 MHz, at 10 and 15 minutes and at 0.3 and 0.5 Watts/cm^2,

(1) between 2 cm and 3 cm ($F_{1, 4}$=81.000, $p<0.01$);

d. at 1 and 3 MHz, at 10 and 15 minutes, and at 0.5 and 0.7 Watts/cm^2,

(1) between skin surface and 1 cm ($F_{1, 4}$=8.162, $p=0.05$);

(2) between 1 cm and 2 cm ($F_{1, 4}$=24.224, $p<0.01$);

e. at 1 and 3 MHz, at 10 and 15 minutes, and at 1.3 and 1.5 Watts/cm^2,

(1) between 1 cm and 2 cm ($F_{1, 4}$=23.198, $p<0.01$);

The contrasts showed that during the initial post-exposure phase (from 10 to 15 minutes), the rate of change in temperatures was greater for the 3 MHz compared with the 1 MHz between adjacent intensities ranging from 0.1 to 0.7, and 1.3 to 1.5 Watts/cm^2, and that these interactions were mainly confined to the superficial tissues of less than 3 cm below the surface.

f. at 1 and 3 MHz, at 15 and 20 minutes and at 0.3 and 0.5 Watts/cm^2,

(1) between 1 cm and 2 cm ($F_{1, 4}$=30.102, $p<0.01$);

g. at 1 and 3 MHz, at 15 and 20 minutes, and at 0.5 and 0.7 Watts/cm^2,

(1) between 3 cm and 4 cm ($F_{1, 4}$=27.842, $p<0.01$).

The contrasts showed that during the late post-exposure phase (from 15 to 20 minutes), the rate of change in temperatures was greater for the 3 MHz compared with the 1 MHz between 0.5 and 0.7 Watts/cm2m, and that these interactions were mainly confined to the middle subcutaneous tissues between 1 and 4 cm below the surface.

Collectively, the contrasts show that as with the lower level interactions already discussed, there is a cumulative effect such that, a higher frequency, a higher intensity, a greater exposure time, and a more superficial site all contribute to a greater increase in temperatures in a cumulative fashion.

No other interactions were significant (Table 8.6).

Table 8.6: Repeated Measures ANOVA (General Linear Model, within subjects effects), and Contrasts (within subjects, repeated) for the four-way interaction effects on main factors (Frequency, Intensity, Duration, Site)

Factor				df	F value	p value	Eta	Power
Freq	**Intensity**	**Duration**	**Site**	105	5.875	<0.01	0.595	1.000
1 vs 3	0.1 vs 0.3	5 vs 10	5 vs 4	1	1.521	0.29	0.276	0.160
			4 vs 3	1	0.035	0.86	0.009	0.052
			3 vs 2	1	0.061	0.82	0.015	0.054
			2 vs 1	1	6.670	0.06	0.625	0.501
			1 vs 0	1	1.619	0.27	0.288	0.167
	0.3 vs 0.5		5 vs 4	1	0.110	0.76	0.027	0.058
			4 vs 3	1	5.939	0.07	0.598	0.459
			3 vs 2	1	0.091	0.78	0.022	0.056
			2 vs 1	1	0.009	0.93	0.002	0.051
			1 vs 0	1	0.875	0.40	0.179	0.113
	0.5 vs 0.7		5 vs 4	1	6.927	0.06	0.634	0.515
			4 vs 3	1	4.618	0.10	0.536	0.377
			3 vs 2	1	0.114	0.75	0.028	0.058
			2 vs 1	1	33.726	<0.01	0.894	0.987
			1 vs 0	1	0.150	0.72	0.036	0.061
	0.7 vs 0.9		5 vs 4	1	0.011	0.92	0.003	0.051
			4 vs 3	1	0.037	0.86	0.009	0.053
			3 vs 2	1	7.237	0.06	0.644	0.532
			2 vs 1	1	4.349	0.11	0.521	0.359
			1 vs 0	1	0.283	0.62	0.066	0.070
	0.9 vs 1.1		5 vs 4	1	1.880	0.24	0.320	0.187
			4 vs 3	1	1.036	0.37	0.206	0.125
			3 vs 2	1	0.852	0.41	0.176	0.111
			2 vs 1	1	0.599	0.48	0.130	0.093
			1 vs 0	1	1.332	0.31	0.250	0.147
	1.1 vs 1.3		5 vs 4	1	0.575	0.49	0.126	0.091
			4 vs 3	1	0.000	0.99	0.000	0.050
			3 vs 2	1	1.913	0.24	0.323	0.189
			2 vs 1	1	0.622	0.47	0.135	0.095
			1 vs 0	1	0.234	0.65	0.055	0.067
	1.3 vs 1.5		5 vs 4	1	0.004	0.95	0.001	0.050
			4 vs 3	1	0.434	0.55	0.098	0.081
			3 vs 2	1	6.180	0.07	0.607	0.473
			2 vs 1	1	4.771	0.09	0.544	0.387
			1 vs 0	1	0.046	0.84	0.011	0.053
	0.1 vs 0.3	**10 vs 15**	5 vs 4	1	0.146	0.72	0.035	0.060
			4 vs 3	1	0.040	0.85	0.010	0.053
			3 vs 2	1	38.252	<0.01	0.905	0.994
			2 vs 1	1	0.078	0.79	0.019	0.056
			1 vs 0	1	0.050	0.83	0.012	0.054
	0.3 vs 0.5		5 vs 4	1	0.073	0.80	0.018	0.055
			4 vs 3	1	0.268	0.63	0.063	0.069
			3 vs 2	1	81.000	<0.01	0.953	1.000
			2 vs 1	1	0.205	0.67	0.049	0.065
			1 vs 0	1	0.593	0.48	0.129	0.093
	0.5 vs 0.7		5 vs 4	1	0.058	0.82	0.014	0.054
			4 vs 3	1	0.334	0.59	0.077	0.074
			3 vs 2	1	0.001	0.98	0.000	0.050
			2 vs 1	1	24.224	<0.01	0.858	0.950
			1 vs 0	1	8.162	0.05	0.671	0.580

Continued on next page

Table 8.6: continued from previous page

0.7 vs 0.9		5 vs 4	1	0.001	0.98	0.000	0.050
		4 vs 3	1	0.000	1.00	0.000	0.050
		3 vs 2	1	0.920	0.39	0.187	0.116
		2 vs 1	1	1.452	0.30	0.266	0.155
		1 vs 0	1	0.341	0.59	0.078	0.074
0.9 vs 1.1		5 vs 4	1	0.058	0.82	0.014	0.054
		4 vs 3	1	0.058	0.82	0.014	0.054
		3 vs 2	1	0.115	0.75	0.028	0.058
		2 vs 1	1	0.329	0.60	0.076	0.074
		1 vs 0	1	6.548	0.06	0.621	0.494
1.1 vs 1.3		5 vs 4	1	0.011	0.92	0.003	0.051
		4 vs 3	1	0.139	0.73	0.034	0.060
		3 vs 2	1	0.895	0.40	0.183	0.115
		2 vs 1	1	1.555	0.28	0.280	0.163
		1 vs 0	1	1.738	0.26	0.303	0.176
1.3 vs 1.5		5 vs 4	1	0.832	0.41	0.172	0.110
		4 vs 3	1	3.574	0.13	0.472	0.307
		3 vs 2	1	3.668	0.13	0.478	0.313
		2 vs 1	1	23.198	<0.01	0.853	0.942
		1 vs 0	1	0.644	0.47	0.139	0.096
0.1 vs 0.3	15 vs 20	5 vs 4	1	0.306	0.61	0.071	0.072
		4 vs 3	1	0.021	0.89	0.005	0.051
		3 vs 2	1	3.477	0.14	0.465	0.300
		2 vs 1	1	2.512	0.19	0.386	0.232
		1 vs 0	1	1.614	0.27	0.288	0.167
0.3 vs 0.5		5 vs 4	1	1.028	0.37	0.204	0.124
		4 vs 3	1	1.503	0.29	0.273	0.159
		3 vs 2	1	1.296	0.32	0.245	0.144
		2 vs 1	1	30.102	<0.01	0.883	0.978
		1 vs 0	1	0.138	0.73	0.033	0.060
0.5 vs 0.7		5 vs 4	1	3.545	0.13	0.470	0.305
		4 vs 3	1	27.842	<0.01	0.874	0.970
		3 vs 2	1	1.235	0.33	0.236	0.139
		2 vs 1	1	2.383	0.20	0.373	0.223
		1 vs 0	1	2.832	0.17	0.415	0.255
0.7 vs 0.9		5 vs 4	1	1.706	0.26	0.299	0.174
		4 vs 3	1	0.130	0.74	0.031	0.059
		3 vs 2	1	0.021	0.89	0.005	0.051
		2 vs 1	1	0.056	0.82	0.014	0.054
		1 vs 0	1	0.157	0.71	0.038	0.061
0.9 vs 1.1		5 vs 4	1	0.161	0.71	0.039	0.061
		4 vs 3	1	0.147	0.72	0.035	0.060
		3 vs 2	1	0.007	0.94	0.002	0.051
		2 vs 1	1	0.002	0.96	0.001	0.050
		1 vs 0	1	0.185	0.69	0.044	0.063
1.1 vs 1.3		5 vs 4	1	2.424	0.20	0.377	0.226
		4 vs 3	1	0.049	0.84	0.012	0.053
		3 vs 2	1	0.006	0.94	0.002	0.050
		2 vs 1	1	0.052	0.83	0.013	0.054
		1 vs 0	1	0.934	0.39	0.189	0.117
1.3 vs 1.5		5 vs 4	1	0.271	0.63	0.064	0.069
		4 vs 3	1	0.470	0.53	0.105	0.084
		3 vs 2	1	0.018	0.90	0.004	0.051
		2 vs 1	1	0.336	0.59	0.077	0.074
		1 vs 0	1	0.770	0.43	0.161	0.105

Freq = Frequency; df = degrees of freedom, Eta = Estimated effect size

Table 8.7: Summary of main results for one-way, two-way, three-way and four-way interactions

	One-way	Two-way					
Factors	**Comments**	**Comments**	**Comments**	**Comments**	**Comments**	**Comments**	**Comments**
Frequency	Δt for 3 MHz was greater than 1Mhz	Δt between 0.1 and 0.3 W/cm^2 was greater for 3 MHz. Conversely, the Δt between 0.5 and 0.7 W/cm^2 was greater for 1 MHz.	Rate of Δt during post-exposure phase was greater for 3 MHz.	Δt was greater for 3 MHz as the distance from the surface increased up to 2 cm below.	During exposure phase, the rate of Δt was greater as intensity increased from 0.1 to 0.9 W/cm^2. During the post-exposure phase, the rate of Δt was greater as intensity increased from 0.1 to 0.5 W/cm^2.	Significant difference in Δt was mainly confined to the lowest and highest intensities, and confined to the superficial tissues.	As duration of exposure and post- exposure increased, the significant interactions occurred more superficially
Intensity	Δt was greater between adjacent intensities as it increased from 0.1 to 0.9, and between 0.9 and 1.5 W/cm^2						
Duration	Δt was different between each adjacent time period from 5 to 20 and between 20 and baseline						
Site	Δt was different between each adjacent site from surface to 5 cm below						

	Three-way				Four-way
Factors	**Comments**	**Comments**	**Comments**	**Comments**	**Comments**
Frequency	As duration increased, the rate of Δt was greater for 3MHz, and this was confined to lower intensities up to 0.7 W/cm^2	As intensity increased from 0.9 to 1.1 W/cm2, and as distance from surface decreased from 3 to 1 cm, the Δt was greater for 3 MHz.	Exposure: rate of Δt increased from 0.1 to 0.9 W/cm^2 from 0 to 3 cm. Post-exposure: rate of Δt increased from 0.1 to 1.5 W/cm^2 from 0 to 5 cm.	As duration of exposure and post-exposure increased, rate of Δt increased was greater for 3 MHz from 0 to 2 cm.	A higher frequency, a higher intensity, a greater exposure time, and a more superficial site all contribute to a greater Δt
Intensity					
Duration					
Site					

Δt: change in temperature

f. Other results

The means and standard deviations for temperature increases at the skin surface and various tissue depths for 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3 and 1.5 Watts/cm^2 are included in Appendix 8, Tables A8-1 to Tables A8-8 for 1 MHz, and Tables A8-9 to Tables A8-16 for 3 MHz. The thermographic isothermic scans of specimens after 10 minutes exposure for 1 MHz and 3 MHz at the various output intensities are also included (Figure A8-1 and Figure A8-2 respectively, Appendix 8).

g. Summary of main results

A synopsis of the main results for the one-way, two-way, three-way and four-way interactions is summarized in Table 8.7.

(1) Frequency and its interactions (Table 8.7)

3 MHz ultrasound produced greater change in temperature than the 1 MHz frequency, and this was confined to the surface tissues (up to 2 cm below surface) and was greater during the post-exposure phase (for 0.1 to 0.9 W/cm^2), compared with the exposure phase (for 0.1 to 0.5 W/cm^2).

(2) Output intensity and its interactions (Table 8.7)

There were significant differences in the amount of heating produced between each adjacent intensity from 0.1 to 0.9 W/cm^2, and between 0.9 and 1.5 W/cm^2. In contrast, there was no difference in the amount of heating produced between each adjacent intensity from 0.9 to 1.3 W/cm^2. These significant differences in the amount of heating produced by the various intensities appeared to be confined to the lowest and highest intensities, and was most noticeable in the superficial tissues (up to 3 cm below surface.

(3) Duration of exposure / post-exposure and its interactions (Table 8.7)

The change in temperatures was significantly different between each of the four time periods (5, 10, 15 and 20 minutes), and between the 20 minutes and baseline. As the duration of exposure and post-exposure increased, the rate of change in temperature was greater for 3 MHz, and this was confined to the superficial tissues (up to 2 cm below).

(4) Site of target tissue and its interactions (Table 8.7)

There was a significant difference in the change in temperatures between each adjacent tissue site from surface to 5 cm below. The differences were most noticeable for the 3 MHz frequency, and for the lowest and highest intensities, and superficial tissue sites (up to 2 cm below).

(5) Main result (Four-way interactions)

A higher frequency, a higher intensity, a greater exposure time, and a more superficial site all contribute to a greater change in temperature.

8.5 DISCUSSION

The main factors of interests in this study were frequency, output intensity, duration of exposure, site of target tissues, and their interactions. A general discussion will precede the discussion on each of the main factors and their interactions.

a. General

The results of this study, for both the 1 and 3 MHz frequencies, were similar to those obtained in previous studies (Chapters 4, 5, and 6), in terms of the pattern of heating and cooling obtained at the various tissue sites, as illustrated in Figure 4.1 (Chapter 4, p107); Figures 5.2 (Chapter 5, p126) and 5.3 (Chapter 5, p127); Figure

6.3 (Chapter 6, p157); and Figures 8.2 and 8.3. This has already been previously described (Chapter 4, pp 112-3; Chapter 5, pp 147-9; Chapter 6, pp 174-175). In summary, for the exposure phase, the mean change in temperature increased as the duration of exposure increased, up to the final 10^{th} minute for both 1 and 3 MHz frequencies. The magnitude of increase was also related to the depth of the target tissue and the output intensity, with peak temperatures decreasing as the depth increased and as the output intensity decreased (Figures 8.2 and 8.3). For the post-exposure phase (11 to 20 minutes), there were two discernible patterns for both 1 and 3 MHz, for the superficial tissues (surface, 1 and 2 cm below surface) and for the deep tissues (3, 4 and 5 cm below surface). At the skin surface, there was a progressive decrease of the temperatures. At 1 and 2 cm below surface, the temperature "leveled off" at around the peak temperature, and remained relatively constant throughout the entire post-exposure phase. For the deep tissues, at 3, 4 and 5 cm below surface, there was a gradual increase in temperature (less than 1°C) over the entire 10 minutes post-exposure phase. The similar results obtained from all four studies suggests that the heating pattern could be considered as consistent and this was unaffected by the output intensity, although the peak temperatures reached for each of the eight intensities were markedly different (Table 8.1). From Figures 8.2 and 8.3, it can be seen that the dispersion of peak temperatures for each of the eight intensities appeared to depend on both the site of the target tissue, as well as the frequencies. The greatest difference in peak temperature was at the skin surface (17.7°C and 26.32°C for 1 and 3 MHz respectively, Table 8.1). Below the skin surface, the difference in peak temperatures between each adjacent site was less pronounced, and decreased as the distance from the skin surface increased (Table 8.1).

b. Frequency and its interactions

For the frequency factor, it was shown that a 3 MHz frequency ultrasound caused greater increase in temperatures compared to 1 MHz (one-way effects, Table 8.7). The two-way interactions (Table 8.7) showed that 3 MHz caused greater increase in temperatures at the superficial sites, up to 2 cm below skin surface (frequency and site), and was greater for the post-exposure phase compared with the exposure phase (frequency and duration). The three-way interactions (Table 8.7) showed that 3 MHz caused greater increase in temperatures for intensities ranging from 0.1 to 0.7 Watts/cm2 (frequency, intensity and duration) and this was confined to superficial sites up to 3 cm below the surface (intensity, duration and site).

At the skin surface, the mean increase in temperatures were approximately 8.5°C and 13.0°C for 1 MHz and 3 MHz respectively (Figure 8.8). Given that the normal resting surface skin temperature ranges from 28°C to 32°C, depending on the subject's activity and environment, and tissue damage starts to occur above 45°C (Stevens 1983), it is possible that increase in surface skin temperatures caused by the 3 MHz frequency ultrasound could be considered unsafe. Although the cooling effect of circulation in the superficial tissues on skin surface temperature is unknown, the possible danger of overheating cannot be totally discounted. When the three-way interactions among frequency, site and intensity are taken into consideration (Figure 8.13), it can be seen that for 3 MHz frequency, the four upper intensities (0.9, 1.1, 1.3 and 1.5 Watts/cm^2) are all capable of increasing the surface skin temperatures greater than 15°C. In contrast, none of the intensities in the 1 MHz frequency produced increases in temperatures beyond 15°C. Hence, it would appear that caution should be exercised when using a 3MHz frequency ultrasound at intensities greater than 0.9 Watts/cm^2.

c. Output intensity and its interactions

For the output intensity factor, there was an almost linear increase in temperatures as the output intensity increased from 0.1 to 1.5 Watts/cm^2 (one-way effects). Again, considering that the core body temperature is around 37°C and that therapeutic effects occur between 38°C and 45°C (Lehman 1971, Lehman and deLateur 1982, Kanui 1987), an increase of at least 1°C can be considered to be clinically useful. From the two-way interactions between output intensity and site of target tissue (Figure 8.10), it can be seen that up to 1 cm below the skin surface any intensity including 0.1 Watt/cm2 would be able to achieve an increase of tissue temperatures greater than 1°C. For target tissues at 2 cm, 3 cm, 4 cm and 5 cm below the skin surface, intensities greater than 0.3, 0.7, 1.1 and 1.5 Watts/cm^2 respectively, would be needed to achieve at least a 1°C rise in tissue temperatures (Figure 8.10). From the three-way interactions among output intensity, site and frequency, and based on the same assumption that a minimum of 1°C rise in tissue temperatures is clinically useful, the 1 MHz frequency ultrasound was able to achieve this up to a depth of 5 cm from the skin surface (Figure 8.13). In contrast, for the 3 MHz frequency ultrasound was only able to achieve this up to a depth of 4 cm (Figure 8.13).

d. Duration of exposure / post-exposure and its interactions

For the duration of exposure and post-exposure factor, it was demonstrated that there was an almost linear increase in tissue temperatures from the first to the tenth minute of exposure, with tissue temperatures peaking at the maximum 10th minute of exposure (Figure 8.4). It is possible that continued exposure of the tissues to the ultrasound energy beyond ten minutes would produce even greater heating, but this would be limited by the high temperatures at the skin surface

(Figure 8.11), particularly for the higher intensities and at 3 MHz frequency (Figures 8.2, 8.3 and 8.9). During the post-exposure phase, it can be seen that the tissue temperatures at the skin surface and 1 cm below decreased gradually but remained above baseline values (Figure 8.4). While monitoring of the tissue temperatures was terminated after 10 minutes post-exposure, it is reasonable to expect that temperatures at the skin surface and 1 cm below will continue to decrease down to its baseline temperature with sufficient time. In contrast, during the post-exposure phase, tissue temperatures at greater than 2cm below the surface either maintained its peak temperature (from maximum exposure at 10 minutes) or continued to increase gradually beyond its 10^{th} minute peak temperature. These two distinctive patterns of heating can be explained by the way heat is being transferred to the tissues during the exposure and post-exposure phases. During the exposure phase, heat is transferred to the tissues by a process known as conversive heating; that is, mechanical vibrations of the molecules caused by the ultrasound is converted to thermal energy due to frictional forces (Low and Reed 2000). This occurs to varying degrees in the tissues, with the superficial tissues being heated more than the deeper tissues. During the post-exposure phase however, the superficial tissues (which have been heated more vigorously than the deeper tissues) give up their heat to the environment, as well as to the deeper adjacent tissues below through a process known as conductive heating. Hence, tissues closer to the surface (up to 1 cm below the surface) readily lose their heat to the environment with a consequent drop in their temperatures. However, the deeper tissues continue to increase their temperatures as a result of conductive heating from the adjacent superficial tissues above them. Hence, it is erroneous to consider the post-exposure phase as a "cooling down" phase. The effect of local circulation on this heating pattern, however, is unknown and should be investigated in future studies. Nevertheless,

depending on the site of the target tissues, an increase in tissue temperatures may be possible even during the post-exposure phase. Again, if the tissue temperatures were monitored beyond the 10 minutes post-exposure phase, it is very likely that all the temperatures would return to baseline.

e. Site of target tissue and its interactions

For the site of the target tissue factor, it can be seen that increase in temperature diminished exponentially as the distance from the surface increased (Figure 8.5). As expected, the tissues at the skin surface and immediately below the surface demonstrated the greatest heating during the exposure phase, and consequently, also the greatest "cooling" during the post-exposure phase. The various interactions involving site of target tissues have already been discussed above.

8.6 CONCLUSION

The results of this study demonstrate that for a desired increase in tissue temperature at a particular target site, varying intensities can be used if the duration is fixed. Alternatively, for a desired increase in tissue temperature at a particular target site and at a specific intensity, the duration of exposure can be varied. The main effects and their interactions on the mean change in temperature for the four main factors; frequency, output intensity, site of target tissue, and duration of exposure / post-exposure, were all found to be significant. Hence, all the null hypotheses for this study were rejected.

CHAPTER NINE

DISCUSSION: AN EVIDENCE-BASED MODEL FOR DETERMINING TREATMENT DOSAGES IN THERAPEUTIC ULTRASOUND

9.1 INTRODUCTION

The series of experiments conducted in this study were directed at providing evidence for the development of a preliminary model for determining treatment dosages in therapeutic ultrasound by examining the relationship between frequency and output intensity of the ultrasound device, the duration of exposure and the site of the target tissue at various distances from the skin surface. In order to develop the research protocol, initial investigations related to the reliability of the measurement devices, that is, the infrared spot thermometer and the infrared video thermography unit (Chapter 4), the movement speed of the transducer (Chapter 5), the size of the treatment area (Chapter 6), and the minimum output intensity at which thermal damage to the cells begin to occur (Chapter 7), were performed as the answers to these issues had not been adequately provided through the literature review. The results of these research protocol-related findings will be discussed, prior to the results of the main findings.

9.2 PROTOCOL-RELATED FINDINGS

a. Reliability

The test-retest reliability of the infrared spot thermometer and the infrared video thermography unit was found to be slightly different. Overall, the former was found to be less reliable than the latter, and this was attributed to their inherent capabilities and machine-related characteristics. For both types of equipment, the exposure phase was found to be less reliable then the post-exposure phase, and this was attributed to differences in the data collection procedures between the two phases. In addition, the ICC scores for the infrared video thermography unit indicated that the reliability at the superficial tissues (1 and 2 cm below skin surface) was not as good as the deeper tissues (3, 4 and 5 cm below skin surface). This was attributed to the deformation of superficial tissues as a result of contact pressure between the transducer and the skin surface, as well as a result of the poor homogeneity of tissues at the superficial sites (fat and muscle composition) compared with the deeper sites (muscle only).

An unplanned analysis of the twenty minutes of data (at one minute intervals) suggested the possibility of reducing the duration factor from 20 to 4. These four periods: 5^{th} and 10^{th} minutes in the exposure phase, and 15^{th} and 20^{th} minutes in the post-exposure phase, were identified based on their high ICC scores that were above the minimum ICC score of 0.60 for moderate reliability as defined by this study (Portney and Watkins 1993). Therefore, these four specific times were considered representative of the exposure phase (5^{th} and 10^{th} minute) and post-exposure phase (15^{th} and 20^{th} minute), and all data analyses in subsequent studies were confined to these four time periods, thus reducing the number of levels for this factor from 20 to four. In this manner, the data analyses for subsequent studies could be simplified considerably without affecting the overall results.

b. Movement speed of the ultrasound transducer.

A common clinical observation was that moving the transducer of the ultrasound device faster could reduce the sensation of hot spots (instantaneous peak temperature) felt by the patient, but this appeared to be not supported by the results of this study. Figures 5.6 (Speed, Site and Duration) and 5.10 (Speed and Site) in Chapter 5 illustrated that the mean peak temperatures at all tissue sites, including the surface skin temperature, was not significantly different no matter how fast the transducer was moved. However, "hot spots" are usually instantaneous rises in temperature that last for a few seconds and are related to the BNR of the transducer. While the BNR of the transducer used in this experiment was relatively low, (1.4:1 and 3.5:1 for the 1 and 3 MHz respectively), it is possible that ultrasonic transducers with higher BNR could still give rise to "hot spots" which can be felt by the patient, and may be reduced by moving the transducer more quickly, as is the common practice in the clinics. In addition, the sensation of "hot spots" could not be measured in this study with post-mortem pig tissues. In human subjects with intact sensory input from the skin, it is still possible for "hot spots" to be felt and reported by the subject even when the BNR of the transducer is considered low. The failure of this study to demonstrate instantaneous rise in peak temperatures or "hot spots" could also be directly related to the methodology, which measured change in temperature at one-minute intervals only, rather than continuous monitoring of the surface skin temperature. Hence, while the main results from this study demonstrated that varying the movement speed of the transducer did not have an effect on the temperature increase within the tissues exposed to ultrasound, up to a depth of 5 cm, regardless of both the frequency of the transducer and duration of exposure / post-exposure, it is probably prudent to move the transducer quickly rather than slowly in order to minimize the sensation of "hot spots" for the subject.

The investigator found that 60 beats/min was too slow, and the likelihood of "hot spots" being felt by the subject was probably higher. On the other hand, 180 beats/min was difficult for the operator to maintain for 10 minutes. The most comfortable speed for moving the ultrasound transducer, while minimizing the likelihood of "hot spots" being felt by the patient, was at 120 beats/min (moderate speed), and this was the recommended speed for subsequent studies as well as for clinical practice.

This study also demonstrated that at 1.0 Watt/cm^2, the 1 MHz transducer appeared to heat tissues at a greater depth than the 3 MHz transducer. This effect was expected and supported by the literature (ter Haar 1987, Low and Reed 2000). However, whether this same relationship holds for other intensities could not be determined by this study alone and was investigated further in the main study (Chapter 8).

c. Size of the treatment area

From the literature review in Chapter 2 on clinical trials in therapeutic ultrasound, none of the studies reported the size of the treatment area as part of the parameters being investigated, or as a factor requiring control. The results of our study demonstrated that smaller treatment sizes appeared to produce greater changes in temperatures at all tissue sites, compared with larger treatment sizes, and the difference between each treatment size was significant. The size of the treatment area, therefore, is an important consideration when clinicians or researchers attempt to estimate the amount of heating at the target tissue, or when attempting to formulate a dosage for treatment. Unless this is reported clearly in any clinical trial, it cannot be assumed that the amount of ultrasonic energy reaching the target tissues is the same, even if other factors such as frequency, output intensity and duration of

exposure had been reported as unchanged between subjects. The effect of a variable treatment size on the poor outcome of some of these studies cannot be underestimated.

The main finding in this study was similar to that of Chan et al (1998) except the magnitude of temperature increase recorded at a depth of 1 cm was substantially greater in the previous study. While Chan et al (1998) reported increases of 8.3°C and 5.0°C for the 2X ERA and 4X ERA treatment sizes after 4 minutes, the current study recorded a maximum of 3.26°C and 1.33°C at the respective treatment sizes after 4 minutes (see Appendix 5, Table A5-1). In the post-mortem pig tissue samples, the cooling effect of circulation described by Chan et al (1998) was absent. If the cooling effect of circulation in the Chan et al (1998) study is discounted, the actual increase in temperature recorded could be 2°C to 3°C greater than reported, thus making it three to four times greater than that reported in our study. This difference in peak temperatures between the two studies could not be accounted for only by the different frequencies used (3 MHz, Chan et al 1998; 1 MHz in the present study). The site of the target tissue in Chan et al's study (1998) was the patellar tendon, which is relatively superficial with the presence of bone directly below the tendon. In contrast, the specimens used in our study were largely skin, fat and muscle tissues to a depth of 5 cm without the presence of any bony structures. It is possible that the reflection of the ultrasound energy back into the patellar tendon, due to the proximity of the bone underlying it, could have caused additional heating of the target tissues at 1 cm depth (Chan et al 1998). In our present study, only tissues without the presence of bone were intentionally selected as specimens in order to eliminate the confounding effect of reflection of the ultrasound energy. Therefore, this difference in tissue specimens could be a primary reason for the greater heating at 1 cm depth reported in Chan et al's study

(1998). Furthermore, tendonous tissues are relatively avascular, and this may also have an impact on the heat removal, resulting in higher temperatures recorded by Chan et al (1998). Both studies demonstrate the 3 MHz results in more heating superficially when compared with 1 MHz. In addition, the results suggested the presence of bone in the tissue specimens could cause greater heating (approximately three to four times) in tissues directly above the bone due to reflection of the ultrasonic energy.

For the development of a preliminary model on dosimetry, the relationship between frequency, output intensity, depth of target tissue and duration of exposure should be investigated without any potential confounding effects. After these fundamental relationships have been clarified adequately, subsequent studies can be carried out to refine the preliminary model. This may include investigations to clarify the presence of bony tissues in the ultrasonic field, and the effects of circulation on the heating pattern, among others. Hence, for the main study which examined the relationship between frequency, output intensity, depth of target tissue and duration of exposure on the heating pattern, it was considered necessary to exclude the confounding effects of bony tissue within the ultrasonic field.

In addition, for consistencies in treatment applications, the treatment size should always be standardized. Based on the results of this study, it is recommended that treatment sizes be standardized to 2X ERA to achieve the greatest depth of heating in the target tissues.

d. Irreversible thermal damage and output intensity

The main aim of this qualitative histological study was to examine the relationship between output intensity (particularly at the higher intensities) and irreversible thermal damage to the exposed tissues, in order to formulate the upper

limit of output intensities that could be safely investigated in the main study. The main assumption was that the maximum machine allowable intensities were not necessarily without risks of causing irreversible thermal injury to the cells, and that for any useful preliminary model on dosimetry to be formulated, it was necessary to exclude intensities that could be considered unsafe for the subject. Since the upper safety limit of output intensities for therapeutic ultrasound was not apparent from the literature review, this issue needed prior clarification as the results had direct implications for the range of intensities that required investigation in the main study.

The use of "hot" post-mortem tissues for this qualitative histological study can be considered controversial. As far as can be determined, no such studies using "hot" post-mortem tissues have been identified. However, from the literature, there appears to be some support for such an investigative model. It has been demonstrated that cell death does not occur immediately upon death of the animal and that cells can remain viable for several hours after death of the animal (Yu et al 1990, Hagenah et al 1993, Babapulle and Jayasundera 1993, D'Armini et al 1995, Hirel et al 1996, Gaudin et al 1996, Jones et al 1997, Songsasen et al 1998, Kuang et al 1998, Laywell et al 1999, Tumanov et al 1999, Huang et al 2000,Verwer et al 2002). Since time was the main factor determining the percentage of viable versus non-viable cells in post-mortem tissues, the entire study had to be completed within a reasonable time "window" so that cell autolysis would not be a confounding factor. Based on information from the literature, and through informal discussions with colleagues from the Department of Anatomy and Cell Biology at Shinshu University, the reasonable "time window" was identified as two to three hours post-mortem. Data collection for this study, using "hot" post-mortem pig tissues was completed within this three hour "time window". However, in order to control for any unforeseen circumstances which could have accelerated the process of autolysis and

rendered our "time window" invalid, an unexposed tissue specimen taken at the end of data collection phase was used as a control specimen. The slide specimens were sent to an independent, blind veterinary pathologist for reporting, and the results showed that except for the 2.0 Watts/cm^2 intensity at 1 MHz, which was the maximum machine allowable for that frequency, all the other specimens including the control were unremarkable. It should be noted that the maximum machine allowable intensity for the 3 MHz frequency was 1.5 Watts/cm^2 for this particular model of therapeutic ultrasound (Omnisound 3000). However, because only one intensity (2.0 Watts/cm^2) of the eight being investigated (including the control specimen) demonstrated irreversible thermal injury to the cells, it could be argued that the "hot" post-mortem tissue model may not be a valid model to detect thermal injury to the cells, and that the results obtained in this study were merely artifactual. Based on the heating pattern observed in the three previous studies on reliability, movement speed of the transducer, and the size of the treatment area, indications were that the "hot" post-mortem tissue model used in this study was valid, but that the thermal energy from the therapeutic ultrasound, even at maximum machine allowable intensities, was insufficient to cause any serious damage to the post-mortem cells. This assumption was tested by repeating the study at even higher thermal energies so that the full range of thermal injuries from first degree to third degree burns could be demonstrated. Obviously, the use of therapeutic ultrasound to repeat this study was not appropriate, as the previous study had already investigated the range of intensities up to the maximum machine allowable. Even other models of therapeutic ultrasound may have been inappropriate, as their maximum machine allowable intensities might not have varied very much from our Omnisound 3000 model. A suitable alternative was to use microwave diathermy to test the assumption, since this particular form of thermal energy is generally

considered to be more vigorous than therapeutic ultrasound (Low and Reed 2000). The results from our microwave study (unpublished data) demonstrated the full range of thermal injuries from first degree to third degree burns as the output intensity increased. The results from both the microwave diathermy and therapeutic ultrasound studies can be considered as sufficient to validate the "hot" post-mortem model as an appropriate model for this type of investigation, and that the reason behind the failure to detect any serious thermal injuries from the therapeutic ultrasound was related to the less than vigorous maximum machine allowable intensities from the therapeutic ultrasound rather than the investigative model.

Based on the main findings of this study, it was recommended that intensities up to 1.5 Watts/cm^2 (for both 1 and 3 MHz) be used for subsequent studies, as well as for clinical practice, at least where the target tissue consists largely of skin, fat and muscle tissue.

9.3 MAIN FINDINGS

The results from the main study demonstrate that the increase in temperature due to absorption of the ultrasonic energy at any of the investigated target sites (up to 5 cm below surface) is related to the ultrasound frequency, intensity and duration of the ultrasound exposure (ter Haar 1987, Williams 1987).

a. Frequency and its interactions

Since high frequencies attenuate more rapidly than lower frequencies (ter Haar 1987), the 1 MHz ultrasound frequency is considered to provide heating for deep tissues, whereas the 3 MHz is considered to provide heating for superficial tissues. The results of this study support this statement, with some qualifications. From Chapter 8, it was shown that the 3 MHz frequency ultrasound caused greater

increase in temperatures compared with the 1 MHz. However, this was only confined to the superficial sites (up to 2 cm below surface). At the deeper tissue sites (greater than 2 cm), 1 MHz ultrasound provided slightly greater heating than 3 MHz (Figure 8.8), but this was not statistically significant. Furthermore, the 3 MHz frequency ultrasound caused overheating of the surface tissues, particularly at intensities greater than 0.5 Watt/cm^2 (see Appendix 8, Table A8-11) and this could be considered inappropriate for clinical applications, as the safety of the patient cannot be assured.

The evidence regarding frequency seems to suggest that compared with the 3 MHz ultrasound, the 1 MHz frequency may be more appropriate for clinical applications as it does not overheat surface tissues, and at the same time, is able to increase the temperatures of target tissues up to a depth of 5 cm.

b. Intensity and its interactions

The intensities selected for investigation in the main study ranged from 0.1 to 1.5 Watts/cm^2 for both 1 and 3 MHz in a stepwise increment of 0.2 Watts/cm^2 (0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5 Watts/cm^2). The choice of an 0.2 Watts/cm^2 rather than an 0.1 Watts/cm^2 increment between each adjacent intensity was made mainly for practical reasons, without compromising too much on the completeness of the data required to formulate a preliminary model on dosage. From the one-way effects of intensity (Figure 8.5), it can be seen that the temperatures increased almost linearly as intensity increased from 0.1 to 1.5 Watts/cm^2. While the lower intensities (below 0.9 Watts/cm2) demonstrated significant differences between each adjacent intensity, the higher intensities from 0.9 to 1.5 Watts/cm2 did not demonstrate this same relationship. However, the actual temperature difference between each adjacent intensity was less than 1°C, with the difference in peak

temperatures between the lowest (0.1 Watt/cm^2) and highest intensity (1.5 Watts/cm^2) being about 4°C (Figure 8.5). Hence, the average difference in temperatures between each of the eight intensities was about 0.5°C. While there was a statistically significant difference between each adjacent intensity, particularly for intensities less than 0.9 Watts/cm2, it can be argued that the small difference in actual temperatures (less than 1°C) may not be clinically relevant. Therefore, while a smaller increment in intensity would have further improved the definition of the data, the results of our study suggested that an increment of 0.2 Watts/cm^2 was reasonable and appropriate.

In human tissues, Williams (1987) estimated the increase in temperature for most soft tissues produced by a 1 MHz ultrasound frequency at an intensity of 1 Watt/cm^2 is about 0.86°C per minute. Since the size of the treatment area, which has been demonstrated to have a direct effect on amount of heating in the tissues, was not specified, this guideline (Williams 1987) may require further clarification. Based on the results of our study for 1 MHz at 1.0 Watt/cm^2 (Chapter 6), Figure 9.1 compares the guideline by Williams (1987) with our actual data obtained for the various treatment sizes (2X, 3X and 4X ERA), and at various tissue sites (surface, and 1, 2, 3, 4 and 5 cm below surface).

From Figure 9.1, it can be seen that the William's (1987) guideline for calculating increase in tissue temperatures closely approximates our data for the 2X ERA treatment size (top figure) at 1 cm below the skin surface only. Any clinical studies that have or intended to use this guideline in formulating treatment intensities for their experimental subjects would not have been able to accurately target tissues greater than 1 cm. In addition, if the treatment area were greater than 2X ERA, any calculations based on this guideline would have been questionable.

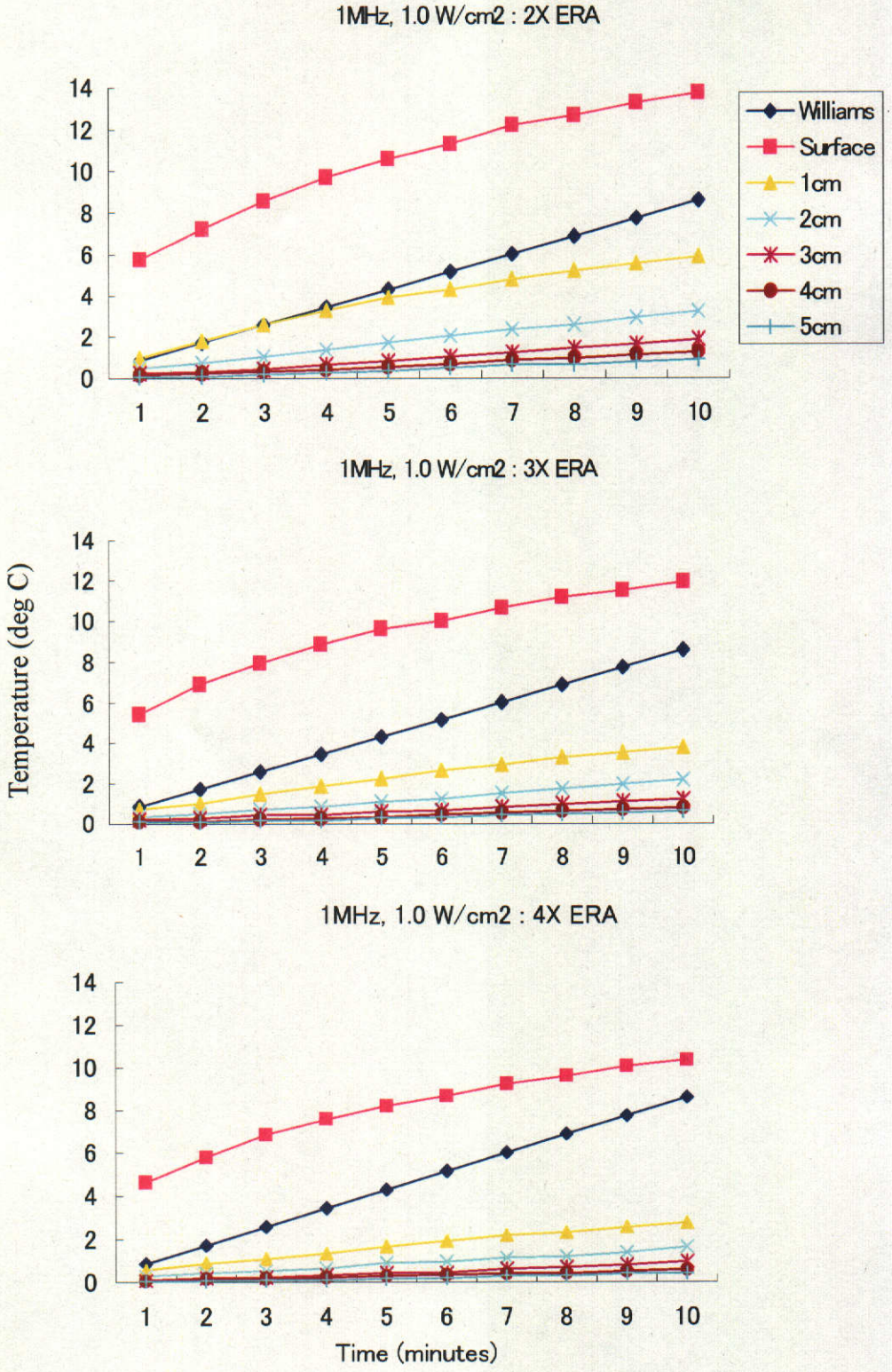

Figure 9.1: Comparison of Williams's (1987) guideline for calculating increase in tissue temperatures (for 1MHz at 1.0 Watt/cm2) and actual data obtained for 2X ERA (top), 3X ERA (middle) and 4X ERA (bottom) size of treatment area.

From our study, it is apparent that the calculation of the treatment intensity is more complicated than just a simple linear equation based on duration of exposure only. Factors that need to be considered in the formula include the size of the treatment area, the site of the target tissue, the output intensity, the frequency of the transducer and the duration of exposure.

Young and Dyson (1990) suggested that the biophysical effects of ultrasound are intensity dependent and that too low a dose will have no effect, while too high a dose will be damaging. While no other guidelines regarding what is too low or too high had been provided by Young and Dyson (1990), the results of our study can be used to partially clarify this statement.

Therapeutic effects are said to occur at temperatures between 40°C and 45°C, maintained for at least five minutes (Lehmann and deLateur 1982). The body's core temperature is usually at 37°C. Hence, an increase of at least 3°C or more in subcutaneous tissues is needed in order for the tissue temperatures to approach the therapeutic range. For 1 MHz therapeutic ultrasound, an increase of 3°C only starts to occur at an intensity of 0.5 Watts/cm^2 (after 10 minutes) at 1 cm below skin surface (see Appendix 8, Table A8-3) and at an intensity of 1.5 Watts/cm^2 (after 20 minutes) at 5 cm below the skin (see Appendix 8, Table A8-8). On the other hand, for 3 MHz therapeutic ultrasound, an increase of 3°C starts to occur at an intensity of 0.3 Watts/cm^2 (after 10 minutes) at 1 cm below the skin (see Appendix 8, Table A8-10) and at an intensity of 1.3 Watts/cm^2 (after 20 minutes) at 3 cm below the skin (Appendix 8, Table A8-15). Even at the highest intensity of 1.5 Watts/cm^2, a 3 MHz ultrasound device is incapable of increasing the tissue temperatures beyond 3°C at 5 cm below the skin in the *in vitro* model used in this series of studies. Assuming that the temperature of the skin surface is around 30°C and that overheating occurs around 45°C, then the limit of temperature increase at the skin surface is about 15°C.

When overheating of the skin surface is taken into consideration, the highest intensity that can be used without exceeding the 15°C increase in surface skin temperature is 1.3 Watts/cm^2 and 0.5 Watts/cm^2 for 1 MHz and 3 MHz respectively. If the aim of the therapeutic ultrasound application is to achieve thermal effects at the target tissues without causing overheating at the surface of the skin, the results of this study suggests that for a 1 MHz frequency device, the dosages for therapeutic effects range from 0.5 to 1.3 Watts/cm^2 for target tissues up to 5 cm. On the other hand, for a 3 MHz frequency device, the dosages for therapeutic effects range from 0.3 to 0.5 Watts/cm^2 for target tissue up to 1 cm only. The minimum exposure time for both frequencies is 10 minutes in order to achieve the desired amount of tissue heating.

Based on the results of our study, the therapeutic range of intensities which can be considered neither too low nor too high (Young and Dyson 1990) are 0.5 to 1.3 Watts/cm^2 and 0.3 to 0.5 Watts/cm^2 for 1 and 3 MHz respectively. Our results also suggest that the narrow therapeutic range for 3 MHz could render it questionable for clinical applications. The larger therapeutic range available for the 1 MHz frequency suggests that it is more suitable for clinical applications and research.

c. Duration of exposure / post-exposure and its interactions

In the absence of concrete guidelines from the literature review, the choice of a 10-minute exposure time for the main study was based on the most commonly used treatment duration in most clinical studies (McDiarmid and Burns 1987). The results of our study suggested this choice was reasonable and appropriate.

Lehmann and deLateur (1982) claimed that therapeutic effects occur at temperatures between 40°C and 45°C maintained for at least five minutes. Assuming a core body temperature of about 37°C, an increase of about 3°C maintained for 5 minutes would be necessary. From the one-way effects of duration

on change in temperature (Figure 8.6, Chapter 8), it can be seen that an increase of 3°C does not occur until after 5 minutes duration of exposure. Hence, if this were maintained for another 5 minutes, based on Lehman and deLateur's (1982) recommendation, the maximum duration of exposure would be 10 minutes.

Oakley (1978) suggested that the duration of the exposure should be related to the size of the treatment area; that is, one to two minutes for each area corresponding to 1.5 times the size of the transducer. For a treatment size of 2X ERA, this would work out to be about 2.7 minutes. Based on our earlier assumption that an increase of at least 3°C or more in subcutaneous tissues is needed in order for the tissue temperatures to approach the therapeutic range, our results demonstrated that after 3 minutes of exposure, except for the skin surface, none of the intensities were capable of achieving an increase of 3°C at any of the tissue sites below the skin surface for both 1 and 3 MHz (see Figures 8.2 and 8.3, Chapter 8). The recommendation by Oakley (1978), therefore, could not be supported by the results of our study.

From pilot studies, the post-exposure phase was found to be more complex than just a "cooling-down" period. The choice of 10 minutes post-exposure phase seems to be supported by the results of this study. The heating pattern seen in this study seems to suggest that there are two types of mechanisms responsible for the increase in tissue temperatures exposed to therapeutic ultrasound. The first mechanism is mainly conversive heating, and this is predominantly in the exposure phase as the mechanical vibrations produced by the ultrasonic energy are converted directly into thermal energy within the tissues, resulting in an increase in tissue temperatures. The second mechanism is through conductive heating, and this is predominantly in the post-exposure phase. When the ultrasonic energy is switched off after ten minutes, the body of heat produced within the tissues in the superficial

layer (up to 2 cm) is conducted away in all directions (upwards, downwards and sideways) to the surrounding tissues. Surface tissues (up to 1 cm) lose heat to the environment more quickly than they gain heat from conductive heating, resulting in a decrease in their temperatures with those near the surface decreasing at a faster rate than those further below the surface. The tissues at the interface between the superficial and deep tissues, as well as the deep tissues continue to gain heat from the conductive heating from the tissues above them. At 2 cm depth, this gain in heat is almost equal to its loss to the surrounding environment, and hence the temperature remains almost unchanged, even after 10 minutes post-exposure. For the deeper tissues at 3 cm, 4 cm and 5 cm, however, this gain is greater than its loss to the surrounding environment, and as such, these tissues continue to increase their temperatures, albeit gradually. The heating pattern of tissues exposed to therapeutic ultrasound, as illustrated in this study, has a major clinical implication. In thermotherapy using therapeutic ultrasound, increase in superficial tissue temperatures can be achieved by exposing the tissues to ultrasonic energy for the desired duration, with the peak temperatures achieved during the exposure phase. At the end of the exposure phase, the tissue temperatures gradually returned to their pre-exposure levels. For deeper tissues at greater depths of more than 2 cm, however, peak temperatures were achieved in the post-exposure phase, and it is essential to recognize that with therapeutic ultrasound, increase in temperatures at target tissues greater than 2 cm depth can be achieved both in the exposure and post-exposure phases. Hence, when the tissues are heated sufficiently to reach the therapeutic range, and then switched off, there is a "time window" of opportunity where the tissues would remain in the therapeutic range, before eventually cooling down and this depended on the maximum temperature reached at the end of exposure, as well as the depth of the target tissue. This "time window" during the

post-exposure phase is useful for implementing certain treatment procedures such as "heat and stretch" (Draper et al, 1998b), and hence the 10 minutes post-exposure period chosen for this study can be considered reasonable. It must be pointed out, however, that effects of local circulation on the heating pattern described are presently unknown and should be investigated in future studies. It is possible that in a highly vascularised treatment area, the cooling effects of local circulation could possibly render the heating pattern of the deeper tissues similar to that of the superficial tissues. In less vascularised treatment areas or where circulation has been compromised, however, the heating pattern for the deep tissues observed in this study could still be a possibility.

d. Site of target tissue and its interactions

When attempting to deliver ultrasonic energy to the target tissues, the depth of the target tissue is a primary consideration. Due to attenuation of the energy, loss of energy occurs as the tissue depth increases (ter Haar 1987). The half value distance for 3 MHz is 1.5 cm and for 1 MHz is 5 cm (Wells 1977). Hence, in order to fully appreciate the heating pattern that occurred within the tissues, a maximum target tissue depth of 5 cm was chosen. The results of our study demonstrated that this choice was reasonable and appropriate. In addition, the distribution of different types of tissue, with their different attenuation characteristics, can also influence the heating pattern produced. Tissues with higher amounts of protein content have been known to absorb ultrasound energy more readily. The specimens used in this study were carefully selected to consist of skin, fat (1 to 2 cm), and muscle (6 to 8 cm), without the presence of any bone, tendons, or ligaments. It is possible that treatment areas with different tissue composition from our specimens could produce different heating profiles, and this should be investigated further in future studies.

From Figure 8.10 (Chapter 8), it can be seen that the change in temperature diminished as the distance from the surface increased. Assuming again that an increase of at least 3°C is necessary in order to reach the therapeutic range, it can be seen that this was achieved up to a depth of about 1 to 2 cm below the skin surface (Figure 8.10, Chapter 8). Hence, the choice of a 5 cm depth for the target tissue in our present study can be considered sufficient.

However, while the other tissue depths at greater than 2 cm only demonstrated an increase of about 1°C, it must be highlighted that the tissue specimens selected for this study did not include any bony structures. In most clinical applications, this is highly unlikely. However, for the formulation of a preliminary model on dosage, it was necessary to exclude any possible confounding variable during the initial investigations. Subsequent investigations, however, should address the issue of reflection of the ultrasonic energy due to the presence of bony structures within the target tissue. From our earlier discussion in relation to the size of the treatment area, a comparison between our data and Chan et al's study (1998) suggested that the presence of bone in the tissue specimens could cause greater heating in tissues directly above the bone due to reflection of the ultrasonic energy by as much as three to four times. Therefore, our present data can be considered as the minimum change in temperatures that can be expected at the desired target site. Hence, while our data suggested that a 1°C increase in temperature can be expected for target depths from 2 to 5 cm below the surface, the presence of any bony structures within these tissues could possibly increase this by three to four times, hence pushing the expected increase in temperature beyond the 3°C increase required to reach the therapeutic range.

Furthermore, there is no evidence to suggest that therapeutic effects are "temperature-specific"; that is, occurring at specific points in the temperature range.

On the contrary, most guidelines (Lehman and deLateur 1982, Kanui 1987) suggest that therapeutic effects occur within a specific temperature range, rather than a specific point. Also, while there is a minimum temperature before therapeutic effects can occur, there is no maximum limit, except for temperatures over 45°C, which would cause skin damage and result in pain felt by the patient (Hardy 1951, Stevens 1983). Therefore, even if the reflection of ultrasonic energy, due to the presence of bony structures within the target tissues in most clinical applications, increases the temperature beyond our estimate from the preliminary model, the model could still be considered clinically useful, as the reflection of the ultrasonic energy would merely push the expected temperature increase further into the therapeutic range.

e. Relationship between Intensity and Duration of Exposure

The energy from the transducer is usually expressed in terms of intensity or power (Low and Reed 2000). Intensity is the amount of energy per unit area, and is usually expressed in Watts/cm^2 (Low and Reed 2000). Power is the total energy in the ultrasonic field across the entire surface area of the transducer, and is usually expressed in watts (Low and Reed 2000). Total power is the power over the entire duration of exposure (that is, power multiplied by time), and is usually expressed in joules (Low and Reed 2000). If the surface area of the transducer is constant, then total power can also be calculated as follows:

a. For continuous mode:

Total Power = intensity (in W/cm^2) X duration (in minutes) X ERA (in cm^2)

b. For pulsed mode:

Total Power = intensity (in W/cm^2) X duration (in minutes) X ERA (in cm^2) X Duty Cycle (in %)

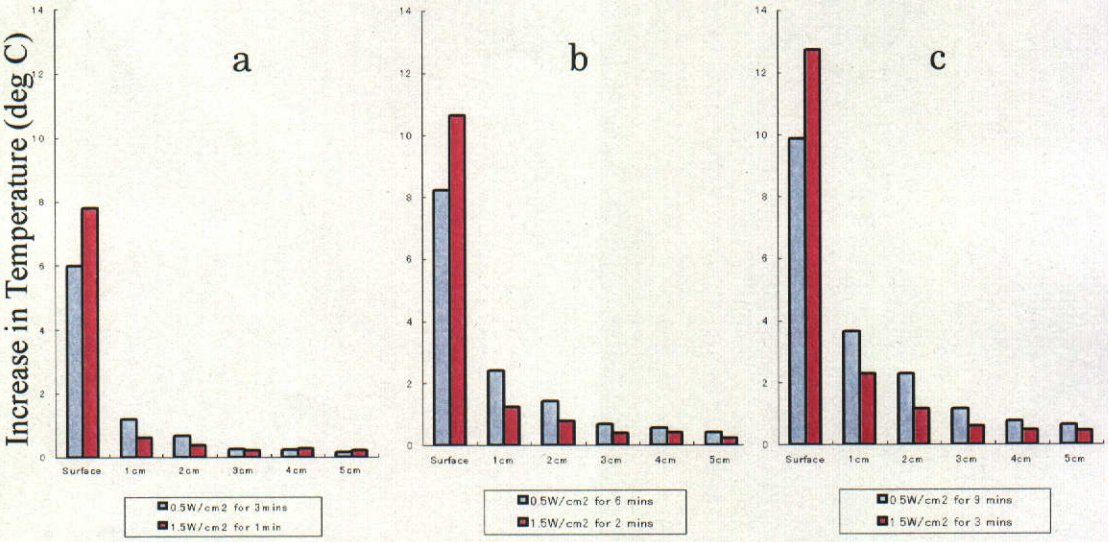

Figure 9.2: Comparison of cumulative effect (total power) for 1MHz ultrasound at (a) 0.5 Watts/cm^2 (3 mins) and 1.5 Watts/cm^2 (1 min); (b) 0.5 Watts/cm^2 (6 mins) and 1.5 Watts/cm^2 (2 mins); and (c) 0.5 Watts/cm^2 (9 mins) and 1.5 Watts/cm^2 (3 min).

Reid and Cummings (1973) suggested that the heating effects of ultrasound are cumulative: that is, 1 Watt/cm^2 for 5 minutes should achieve the same amount of heating as 0.5 Watts/cm^2 for 10 minutes. The total power for both are equivalent, and hence, theoretically, the amount of heating achieved in the tissues should be similar. The eight intensities that were being investigated were 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3 and 1.5 Watts/cm^2. Hence, to examine whether the effects of ultrasound are cumulative, the intensities of 0.5 and 1.5 Watts/cm^2 can be compared. Figure 9.2 compares the cumulative effect of 1 MHz ultrasound at 0.5 and 1.5 Watts/cm^2 for 3 and 1 minutes, 6 and 2 minutes, and 9 and 3 minutes respectively.

From Figure 9.2, it can be seen that while the results show that the two intensities at an equivalent total power do not achieve the same increase in temperatures, they do however, demonstrate some similarities. At the skin surface, the difference is about 2°C, but subcutaneously, the difference is only 1°C or less. For most applications, this difference subcutaneously may not be clinically relevant. Therefore, to a certain extent, the heating effects of therapeutic ultrasound can be

considered to be cumulative. The usefulness of developing a preliminary model on dosimetry based on one variable (total power), rather than two variables (intensity and duration), should be explored in future studies aimed at refining the model. In addition, the effect of varying the duty factor on the heating patterns should also be investigated. Preliminary experiments (unpublished data) conducted at 1MHz frequency for 10 minutes exposure at similar total power output, (1.0 W/cm^2 at 100% duty factor, and 0.25 W/cm^2 at 25% duty factor) showed that the heating pattern were similar for both. Hence, it is possible that if total power, which takes into account the intensity, duration of exposure and duty factor, was used to define the output of the ultrasound energy rather than the three variables separately (intensity, duration and duty factor), the issue of dosimetry in therapeutic ultrasound could be simplified considerably. However, based on the present data, it is not possible to conclusively verify the claims by Reid and Cummings (1973). The proposed preliminary model from this study, therefore, may need to be based on the two separate variables (intensity and duration) until more information clarifying the relationship between total power and change in tissue temperature becomes available.

f. Relationship among Frequency, Intensity, Duration of Exposure and Site of Target Tissue.

The results of our main study demonstrated that a higher frequency, a higher intensity, a greater exposure time and a more superficial site all contribute to a greater change in temperature. While comparable data from the literature is limited and incomplete, a limited comparison is possible and this is summarized in Tables 9.1 and 9.2 for 1 and 3 MHz respectively. A comparison of the results indicated that with one exception (Draper et al 1995b), there were remarkable similarities (within mean±1SD) in the reported change in temperatures for the corresponding intensity and tissue site (indicated by bold fonts in Tables 9.1 and 9.2).

Table 9.1: Comparison of increase in subcutaneous temperature after 10 minutes exposure to 1 MHz ultrasound at various intensities (size of treatment area 2X ERA) (*red italics* indicate data from present study; bold fonts indicate similar results within mean±1SD)

		Increase in temperature (°C) after 10 minutes exposure to ultrasound at corresponding depth of tissue from the surface (in cm)						
Authors	Intensity	1.0 cm	1.5 cm	2.0 cm	2.5 cm	3.0 cm	4.0 cm	5.0 cm
Draper et al 1995a	0.5				0.40*			**0.60***
Mean (SD)				*2.53 (1.50)*		*1.35 (0.48)*		*0.72 (0.14)*
Draper et al 1995a	1.0				**1.60***			1.60*
Mean (SD)				*2.77 (1.07)*		*1.79 (0.82)*		*0.65 (0.63)*
Draper and Sunderland 1993	1.5					**4.90**		
Draper et al 1993						**4.80**		
Draper et al 1995b								4.00
Draper et al 1998a		3.50				**3.85**		
Draper et al 1995a					**3.30***			3.30*
Rimington 1994						**2.00**		
Mean (SD)		*7.19 (0.88)*		*4.70 (1.54)*		*3.36 (1.42)*		*2.05 (1.09)*

* based on an estimate of the reported rate of increase per minute

Table 9.2: Comparison of increase in subcutaneous temperature after 10 minutes exposure to 3 MHz ultrasound at various intensities (size of treatment area 2X ERA) (*red italics* indicate data from present study; bold fonts indicate similar results within mean±1SD)

		Increase in temperature (°C) after 10 minutes exposure to ultrasound at corresponding depth of tissue from the surface (in cm)						
Authors	Intensity	1.0 cm	1.5cm	2.0 cm	2.5 cm	3.0 cm	4.0 cm	5.0 cm
Draper et al 1995a	0.5	**3.00***	3.00*					
Mean (SD)		*3.95 (0.95)*						
Draper et al 1995a	1.0	**5.80***	5.80*					
Levine et al 2001**		**3.00**		**2.30**		**1.60**		
Myrer et al 2001		**7.47**						
Mean (SD)		*5.78 (2.77)*		*2.70 (2.02)*		*1.74 (1.43)*		
Draper et al 1995a	1.5	**8.90***	8.90*					
Levine et al 2001**		4.60		**3.60**		**2.40**		
Mean (SD)		*8.03 (1.07)*		*3.54 (0.95)*		*2.23 (0.62)*		

* based on an estimate of the reported rate of increase per minute; ** 3.3MHz frequency

The results from Levine et al's study (2001), however, showed remarkable similarities to our results, especially at 2 and 3 cm, despite using a slightly different frequency (3.3 MHz).

The only study that differed from our results was different by merely 1°C (Draper et al 1995b). Based on the limited comparisons between the results of our study and *in vivo* studies reported in the literature, the remarkable similarities could be an indication that the effect of circulation, which is absent from our study, could be minimized by using the temperature ± a variance of one standard deviation. The overall cooling effect of circulation on the heating pattern was reported to be about –1°C to –3°C (Lehmann et al 1966b). The variance in our results was within ±2°C, and is consistent with the range reported by Lehman et al (1966b). Interestingly, the results from our study were usually (but not always) greater than that reported by the *in vivo* studies, suggesting that the differences were due to the cooling effect of circulation in the *in vivo* studies, and which was absent from our study.

9.4 A RE-EVALUATION OF CLINICAL STUDIES IN LIGHT OF MAIN FINDINGS

Based on the main findings of our study, a re-evaluation of the clinical studies reviewed in the literature survey (Chapter 2) can be attempted in relation to the appropriateness of the dosage selected for investigation in these studies. In summary, the main findings from our study suggests that:

a. Compared with the 3 MHz ultrasound, the 1 MHz frequency may be more appropriate for clinical applications as it does not overheat surface tissues, and at the same time, is able to increase the temperatures of target tissues up to a depth of 5 cm.

b. The calculation of the treatment intensity is more complicated than just a

simple linear equation based on duration of exposure only. Factors that need to be considered in the formula include the size of the treatment area, the site of the target tissue, the output intensity, the frequency of the transducer and the duration of exposure. The site of the target tissue can be reasonably estimated from a sound knowledge of anatomy and pathology. While parameters such as the output intensity, frequency and duration of exposure are usually specified in most clinical studies, the size of the treatment area, on the other hand, is rarely reported adequately. This can explain why studies using similar parameters such as output intensity, frequency and duration of exposure, but unknown and presumably different treatment sizes, can report different outcomes.

c. The therapeutic range of intensities, which can be considered neither too low nor too high (Young and Dyson 1990) are 0.5 to 1.3 Watts/cm^2 and 0.3 to 0.5 Watts/cm^2 for 1 and 3 MHz respectively.

d. The minimum duration of exposure when subcutaneous tissues begin to approach the therapeutic range is five minutes. Based on Lehman and deLateur's (1982) claim that therapeutic effects begin to occur only after maintaining the temperature at the therapeutic range for another 5 minutes, the optimum duration of exposure should be around 10 minutes. The initial 5 minutes (approaching therapeutic range) may be shortened in cases where the treatment area is small (less than 2X ERA), or the site of the tissue is very superficial (less than 1 cm below skin surface), or when there is a bony structure underlying the target tissue which can increase the rate of heating through reflection of the ultrasonic energy back into the target tissues.

Table 9.3: Summary of Phase 2 RCTs investigating the effect of ultrasound on inflammation

Authors	Diagnosis (No. of subjects)	Frequency / Duration / Intensity (W/cm^2)	Outcome of study and Comments	Ability to predict outcome
Nyanzi et al 1999*	Acute ankle sprains (26 Exp vs 25 Pla)	3 MHz / 10 mins / Pulsed 20%, SATP 0.25, SATA 0.05	No significant difference between groups. Less than therapeutic range for 3 MHz	True negative
Downing & Weinstein 1986*	Subacromial bursitis (11 Exp vs 9 Pla)	1 MHz / 6 mins / Cont, SATP variable (mean 1.2)	No significant difference between groups. Unable to assess the appropriateness of dosage due to variable dosages.	Unknown
Nykanen M 1995*	Rotator cuff syndrome (35 Exp vs 37 Pla)	1 MHz / 10 mins / Pulsed 20%, SATP 1.0, SATA 0.2	Both groups improved, but no significant difference between groups. Less than therapeutic range for 1 MHz.	True negative
Ebenbichler et al 1999*	Calcific tendonitis Shoulder (32 Exp vs 29 Pla)	0.89 MHz / 15 mins / Pulsed 20%, 2.5, SATA 0.5	Exp group improved significantly. Within therapeutic range for 1 MHz.	True positive
Van der Heijden et al 1999*	Shoulder pain (39 Exp vs 33 Pla vs 35 Con)	0.8 MHz / > 2 mins / Pulsed 20%, SATP variable	No significant difference between groups. Unable to assess the appropriateness of dosage due to variable dosages used.	Unknown
Binder et al 1985*	Lateral epicondylitis (25 Exp vs 23 Pla)	1 MHz / 5 to 10 mins / Pulsed 20%, SATP 1.0 to 2.0, SATA 0.2 to 0.4	Exp group improved significantly. Approaching therapeutic range for 1 MHz, but still slightly lower. The superficial nature of the lesion (with the presence of bone below the target tissue), in addition to the localized and small treatment area could have helped to push the temperatures into the therapeutic range	True positive
Lundeberg et al 1988*	Lateral epicondylitis (33 Exp vs 33 Pla vs 33 Con)	1 MHz / 10 mins / Cont, SATP 1.0	Exp group significantly better than Cont group, but no difference between Exp and Pla. Within therapeutic range for 1 MHz.	False negative
Haker & Lundeberg 1991*	Lateral epicondylitis (21 Exp vs 22 Pla)	1 MHz / 10 mins / Pulsed 20%, SATP 1.0, SATA 0.2	No significant difference between groups. Less than therapeutic range for 1 MHz.	True negative
ElHag et al 1985*	Molar extraction (33 Exp vs 32 Pla)	3 MHz / 8 mins Pulsed 20%, SATP 0.5, SATA 0.1	Exp group improved significantly. Less than therapeutic range for 3 MHz.	False positive
Hashish et al 1986*	Molar extraction (25 Exp vs 25 Pla vs 25 Con)	3 MHz / 5 mins / Pulsed 20%, SATP 0.1, 0.5, 1.5, SATA 0.02, 0.1, 0.3	Exp and Pla groups significantly better than controls. Within therapeutic range for 3 MHz, but short treatment duration could be a confounding variable.	True positive
Hashish et al 1988*	Molar extraction (25 Exp vs 25 Pla vs 50 Con)	3 MHz / 5 mins / Pulsed 20%, SATP 0.1, SATA 0.02, SATA 0.02, 0.1, 0.3	Exp and Pla groups significantly better than controls. Within therapeutic range for 3 MHz, but short treatment duration could be a confounding variable.	True positive

*Subjects were randomized into groups.
Exp = Experimental group; Pla = Placebo group; Con = Control group; SATP = Spatial Averaged Temporal Peak; SATA = Spatial Averaged Temporal Average; Cont = Continuous

Table 9.4: Summary of Phase 2 RCTs investigating the effect of ultrasound for pain relief

Authors	Diagnosis	Frequency / Duration / Intensity (W/cm^2)	Outcome	Ability to predict outcome
Creates 1987*	Painful perineum (39 Exp vs 37 Pla)	1 & 3 MHz / according to PT / Pulsed, according to PT	Exp group improved significantly. Unable to assess appropriateness of dosage due to variable frequency, intensity and duration	Unknown
Grant et al 1989*	Perineal trauma (140 Exp vs 139 Pla)	3 MHz / 6 to 18 mins / Pulsed 20%, SATP 0.5, SATA 0.1	No significant difference between groups. Less than therapeutic range for 3 MHz.	True negative
Everett et al 1992*	Perineal pain (37 Exp vs 32 Pla)	3 MHz / 5 mins / Pulsed 50%, SATP 0.5, SATA 0.25	Exp group had favorable outcome, but this was not significant. Less than therapeutic range for 3 MHz.	True negative
Nwuga 1983*	Prolapsed Intervertebral disc (27 Exp vs 25 Pla vs 29 Con)	? MHz / 10 mins / Cont, SATP 1.0 to 2.0	Exp group improved significantly. Unable to assess appropriateness of dosage due to insufficient information regarding frequency.	Unknown
Inaba & Piorkowski 1972*	Painful shoulders in hemiplegia (10 Exp vs 10 Pla vs 13 Con)	? MHz / 10 mins / Cont, SATP 0.5 to 2.0	No significant difference between groups. Unable to assess appropriateness of dosage due to insufficient information regarding frequency.	Unknown
Gam et al 1998*	Myofascial pain (18 Exp vs 22 Pla vs 18 Con)	0.1 MHz / 3 to 15 mins / Pulsed 20%, SATP 3.0, SATA 0.6	Exp and Pla groups significantly better than Con, but no difference between Exp and Pla. Unable to assess appropriateness of dosage due to unique frequency.	Unknown
Crawford & Snaith 1996*	Heel pain (13 Exp vs 13 Pla)	3 MHz / 8 mins / Pulsed 20%, SATP 0.5, SATA 0.1	Exp group had favorable outcome, but this was not significant. Less than therapeutic range for 3 MHz.	True negative
Gray et al 1995)*	TMJ pain (30 Exp vs 26 Pla)	3 MHz / 2 mins / Pulsed 66%, SATP 0.25, SATA 0.16	Exp group "improved", but no statistical analysis due to small sample size (according to author). Less than therapeutic range for 3 MHz.	Unknown
Hasson et al 1990*	DOMS (6 Exp vs 6 Pla vs 6 Con)	1 MHz / 20 mins / Pulsed 20%, SATP 0.8, SATA 0.16	Exp was significantly better than Pla or Cont. Less than therapeutic range for 1 MHz, but long treatment duration could have pushed the temperatures into the therapeutic range.	True positive
Stay et al 1998*	DOMS (12 Exp vs 12 Pla)	1 MHz / 7 mins / Pulsed 20%, SATP 1.5, SATA 0.3	No significant difference between groups. Less than therapeutic range for 1 MHz.	True negative
Plaskett et al 1999*	DOMS (10 Exp vs 10 Pla)	1 MHz / 8 mins / Pulsed 20%, SATP 1.0, SATA 0.2	No significant difference between groups. Less than therapeutic range for 1 MHz.	True negative
Craig et al 1999*	DOMS (12 Exp vs 12 Pla vs 12 Con)	1 MHz / 7 & 15 mins / Pulsed 20%, SATP 0.8, SATA 0.16	No significant difference between groups. Less than therapeutic range for 1 MHz.	True negative

*Subjects were randomized into groups.

Exp = Experimental group; Pla = Placebo group; Con = Control group; SATP = Spatial Averaged Temporal Peak; SATA = Spatial Averaged Temporal Averaged; Cont = Continuous, DOMS = Delayed onset muscle soreness

Table 9.5: Summary of Phase 2 RCTs investigating the effect of ultrasound on tissue healing

Authors	Diagnosis	Frequency / Duration / Intensity (W/cm^2)	Outcome	Ability to predict outcome
Eben-bichler et al 1998*	Carpal tunnel syndrome (34 Exp vs 34 Pla)	1 MHz / 15 mins / Pulsed 20%, SATP 1.0, SATA 0.2	Exp group improved significantly. Less than therapeutic range for 1 MHz, but long treatment duration could have pushed the temperatures into the therapeutic range.	True positive
Oztas et al 1998*	Carpal tunnel syndrome (10 Exp vs 10 Pla)	3 MHz / 5 mins / Cont, SATP 0.8 & 1.5	Exp group had favorable outcome, but this was not significant. More than therapeutic range for 3 MHz.	True negative
Dyson & Suck-ling 1978*	Venous ulcers (7 Exp vs 7 Pla)	3 MHz / 5 to 10 mins / Pulsed 20%, SATP 1.0, SATA 0.2	Exp group improved significantly compared to placebo. Less than therapeutic range for 1 MHz, but the absence of skin over the target tissue could have pushed the temperatures into the therapeutic range.	True positive
Roche & West 1984*	Venous ulcers (13 Exp vs 13 Pla)	3 MHz / 5 to 10 mins / Pulsed 20%, SATP 1.0, SATA 0.2	Exp group improved significantly compared to placebo. Same as above.	True positive
Callam et al 1987*	Chronic leg ulcers (52 Exp vs 56 Con)	1 MHz / 1 minute per probe area / Pulsed 10%, SATP 0.5, SATA 0.05	Exp group improved significantly compared to controls. Same as above	True positive
Weich-enthal et al 1997*	Chronic venous ulcers (19 Exp vs 18 Con)	30 kHz / 10 mins / Cont, SATP 0.01	Exp group improved significantly compared to controls. Same as above	True positive
Lunde-berg et al 1990*	Venous ulcers (22 Exp vs 22 Pla)	1 MHz / 10 mins / Pulsed 10%, SATP 0.5, SATA 0.05	No significant difference between groups, but tendency for Exp group to improve more than Pla. Unable to assess the appropriateness of dosage due to the inclusion of a standard wound treatment protocol for both groups could have confounded the results.	Unknown
Eriksson et al 1991*	Chronic leg ulcers (19 Exp vs 19 Pla)	1 MHz / 10 mins / Cont, SATP 1.0,	No significant difference between groups. Same as above.	Unknown
ter Riet et al 1996*	Pressure ulcers (45 Exp vs 43 Pla)	3.28 MHz / 3 to 10 mins / Pulsed 20%, SATP 0.5, SATA 0.01	No significant difference between groups. Same as above.	Unknown

*Subjects were randomized into groups.
Exp = Experimental group; Pla = Placebo group; Con = Control group; SATP = Spatial Averaged Temporal Peak; SATA = Spatial Averaged Temporal Averaged; Cont = Continuous

Table 9.6: Summary of Phase 2 RCTs investigating the effect of ultrasound on tissue extensibility

Authors	Diagnosis	Frequency / Duration	Intensity (W/cm^2)	Outcome
Falconer et al 1992*	OA Knee (34 Exp vs 35 Con)	1 MHz / 12 mins / Cont, SATP variable	No difference between groups. Unable to assess appropriateness of dosage due to variable intensity..	Unknown
Ward et al 1994*	Scar contracture (8 Exp vs 6 Pla)	1 MHz / 10 mins / Cont, SATP 1.0	No difference between groups. Both groups received stretching exercises, and the dependent variable was ROM. Unable to assess appropriateness of dosage due to poor study design.	Unknown

*Subjects were randomized into groups.
Exp = Experimental group; Pla = Placebo group; Con = Control group; SATP = Spatial Averaged Temporal Peak; Cont = Continuous

With these guidelines for determining treatment dosages in mind, a re-evaluation of the clinical studies reviewed in the literature survey is summarized in Tables 9.3, 9.4, 9.5 and 9.6. The aim of the re-evaluation of the clinical study was to assess the appropriateness of the treatment dosages selected for the study, based on the preliminary guidelines formulated from the results of our own study. A secondary aim was to assess the ability of our preliminary guidelines to correctly predict the outcome of the study as follows:

a. "True positives": Studies that had a **positive outcome** and whose treatment dosages were also **deemed appropriate**.

b. "True negatives": Studies that had a **negative outcome** and whose treatment dosages were **deemed NOT appropriate**.

c. "False positives": Studies that had a **positive outcome** and whose treatment dosages were **deemed NOT appropriate**.

d. "False negative": Studies that had a **negative outcome** and whose treatment dosages were **deemed appropriate**.

e. "Unknown": Unable to make a judgement due to limited information.

a. Summary

A total of 34 clinical studies (Tables 9.3 to 9.6) were re-evaluated from the perspective of assessing the appropriateness of the dosages being investigated. The main conclusions from this re-evaluation can be summarized as follows::

a. "True positives" (indicated by comments in green): Studies with positive outcomes that employed appropriate dosage parameters that were considered by the results of this study to be within the therapeutic range (10 out of 34 studies, or 29%). Interestingly, 4 of these clinical studies were related to the effect of ultrasound on inflammation, and five were related to tissue healing, suggesting that these could be the recommended dosages for treatment of inflammation and tissue healing.

b. "True negatives" (indicated by comments in blue): Studies with negative outcomes that employed inappropriate dosage parameters that were considered by the results of this study to be lower or greater than the therapeutic range (10 out of 34 studies, or 29%).

c. "False positives" (indicated by comments in red): Studies with positive outcomes that employed inappropriate dosage parameters that were considered by the results of this study to be lower or higher than the therapeutic range (1 out of 34 studies, or 3%).

d. "False negatives" (indicated by comments in brown): Studies with negative outcomes that employed dosage parameters that were considered by the results of this study to be appropriate or within the therapeutic range (1 out of 34 studies, or 3%)

e. "Unknown" (indicated by comments in black): Studies with either positive or negative outcomes but were unable to assess the appropriateness of the dosage parameters due to insufficient information (12 out of 34 studies, or

35%).

When the studies with insufficient information to formulate a judgement on their treatment dosages were excluded, the percentage of true positives, true negatives, false positives and false negatives increased to 45%, 45%, 5% and 5% respectively. Hence, the accuracy of predicting a true positive or a true negative outcome by using the guidelines based on the results of this study is about 90%. This would strongly suggest that a preliminary model to determine treatment dosages based on the results of this study is highly possible.

9.5. THE DEVELOPMENT OF A PRELIMINARY MODEL FOR DETERMINING TREATMENT DOSAGES IN THERAPEUTIC ULTRASOUND

A re-evaluation of the clinical studies demonstrated that improper selection of treatment dosages could be a major contributing factor to negative outcomes. Despite the large number of clinical studies on therapeutic ultrasound published to date, the determination of treatment dosages remains largely empirical (Reid and Cummings 1973, Goh et al 1999). The lack of a common model to determine treatment dosages could have been a significant factor affecting the results of clinical trials. This factor emphasizes the need to develop a common model for determining treatment dosages that can be used to guide both clinicians and researchers in the use of therapeutic ultrasound.

The development of a model for determining treatment dosages in therapeutic ultrasound can be considered as a two-stage process. The first stage involves the ability to consistently deliver a fixed amount of ultrasonic energy to the target tissue. This can be achieved by having a thorough understanding of the relationship between the frequency, output intensity and duration of exposure of the

ultrasonic energy on temperature increase at various distances from the skin surface. The second stage involves understanding the effect of this energy on the target pathological tissue. This can be achieved by using the information from the first stage to conduct clinical trials on various patient populations and to adjust the amount of ultrasonic energy to the target pathological tissue based on its response to the treatment. Through this two-stage process, it is possible to provide the evidence for the clinical effectiveness of therapeutic ultrasound.

Using the electrophysical agents (EPA) research model proposed in Figure 2.2 (Chapter 2), the first stage in the development of a preliminary model for determining treatment dosages in therapeutic ultrasound would involve initial cellular and animal testing and preliminary human testing (Figure 2.2). The series of studies carried out in this thesis would be classified under this category. While only cellular and animal studies on post-mortem pig tissues have been carried out, a limited comparison of the results obtained through these studies with other *in vivo* studies using animal and human subjects (Tables 9.1 and 9.2) indicated that the obtained *in vitro* data was comparable with the results from *in vivo* studies. This might suggest that the use of an experimental *in vitro* animal model might be a reasonable first step in establishing relationships between the various parameters necessary for formulating a dosimetry model, and that the data obtained through this kind of investigations may be comparable to data obtained from *in vivo* human subject testing. While it does not mean that the latter can be excluded entirely, *in vivo* human subject testing can be minimized and perhaps restricted to validating the results obtained from the initial animal studies. The ethical implications of using such an investigative model for research in electrophysical agents is obvious, and could possibly contribute significantly to our future research efforts in the area of electrophysical agents.

The second stage in the development of a preliminary model for determining treatment dosages in therapeutic ultrasound would involve Phase 1, 2 and 3 studies on relevant patient populations. The adoption of the preliminary model to determine treatment dosages in these Phase 1, 2 and 3 studies would enable researchers to perform cross comparisons of the results from these studies, and hence, facilitate the integration of these data into the preliminary model. In this way, the preliminary model can be refined and improved upon until it becomes robust enough to be used as a standard model for determining treatment dosages for therapeutic ultrasound, both clinically and in research. Hence, there is an obvious need for a model to assist clinicians and researchers in determining treatment dosages based on experimental evidence of the expected increase in temperatures at desired target sites. In order for any useful model to be derived from the results our studies, the proposed preliminary model should fulfill the following criteria and guidelines:

a. **Simplicity:** As the model is intended for both clinical and research purposes, it should be as simple as possible in order to facilitate its usage. The information should be as succinct as possible, with only the relevant data or parameters being chosen for inclusion in the model.

b. **Completeness:** In order to cater for all types of treatment application, the data included in the model must be complete. Clinicians and researchers require guidelines for all types of treatment applications, from acute to chronic conditions, and hence, any gaps in the information would render the model useless.

c. **Consistency:** In addition to providing guidelines for selecting treatment dosages for various conditions, it is also necessary to provide information on how to minimize inconsistencies in the treatment procedure, which can have an adverse effect on the treatment application. This procedural information,

while not considered as part of the model itself, can be provided as notes to the clinician or researcher to ensure that they do not deviate from the recommended treatment procedure.

Base on the above criteria as well as the results of our studies, a model for assisting clinicians and researchers to select treatment dosages based on desired increase in tissue temperatures at the target site (up to a depth of 5 cm below the skin surface) can be proposed. The evidence from our series of studies seems to suggest that the relevant parameters to be considered when selecting a treatment dosage for therapeutic ultrasound are:

a. **Frequency:** The need to have two frequencies (1 and 3 MHz) in the proposed model requires careful examination. As far as can be determined, the tissue's response to the ultrasound energy is not frequency specific. The often-cited reason for using 1 and 3 MHz is that high frequencies attenuate more rapidly than lower frequencies (ter Haar 1987). Hence, the 1 MHz ultrasound frequency is considered to provide heating for deep tissues, whereas the 3 MHz is considered to provide heating for superficial tissues. However, 1 MHz ultrasound devices, while being able to provide heating for deep tissues, can also be selected to provide heating for superficial tissues. On the other hand, the 3 MHz ultrasound device is only capable of providing heating for superficial tissues. Furthermore, our studies show that the problem with the 3 MHz ultrasound device is that it can easily cause overheating of the superficial tissues, without being able to increase the subcutaneous tissues up to the required therapeutic range. Therefore, compared with the 3 MHz ultrasound, the 1 MHz frequency may be more appropriate for clinical applications as it does not overheat surface tissues, and at the same time, is able to increase the temperatures of target

tissues up to a depth of 5 cm. Hence, it is proposed that the 3 MHz frequency be excluded from the dosimetry model, thus limiting the frequency parameter to 1 MHz only. This would simplify the model considerably, without limiting its usefulness.

b. **Intensity:** The results of our study suggests that the therapeutic range of intensities which could be considered neither too low as to be ineffective, nor too high as to be damaging to the tissues (Young and Dyson 1990) are 0.5 to 1.3 Watts/cm^2, and 0.3 to 0.5 Watts/cm^2 for 1 and 3 MHz respectively. Again, the narrow range of therapeutic intensities for the 3 MHz frequency in comparison to the 1 MHz frequency suggests that the former could be excluded from the proposed model. The results of our study also indicate that intensities beyond 1.5 Watts/cm^2 should be excluded since there was some evidence that high intensities could cause irreversible thermal injury to the cells (Chapter 7). The intensities, therefore, that should be included in the model should range from 0.1 to 1.5 Watts/cm^2 in a stepwise increment of 0.2 Watts/cm^2; that is, 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5 Watts/cm^2 (see Appendix 8, Tables A8-1 to A8-8). In addition, as clinicians often use 1.0 Watts/cm^2 as a convenient treatment intensity, it should also be included in the model (see Appendix 5, Tables A5-1 to A5-3). Hence, the model will provide information for nine treatment intensities for 1 MHz frequency.

c. **Duration:** Since the heating effects of therapeutic ultrasound can be considered cumulative to a certain extent, the two parameters of duration and intensity are related by its total power, which is defined as intensity (in watts) multiplied by the duration of exposure (in minutes). If the duration factor is held constant, then the only variable that needs to be considered by

the clinician or researcher is the intensity parameter. This would again simplify the model considerably. The results of our study indicate that the optimum time to achieve adequate heating of the deep tissues (up to a depth of 5 cm) without causing overheating of the surface tissues is 10 minutes. Therefore, it is proposed that the duration factor be standardised to 10 minutes in the dosimetry model.

d. **Site of Target Tissue:** Assuming that an increase of at least 3°C is necessary in order to reach the therapeutic range, our results suggests that this can be achieved up to a depth of about 1 to 2 cm below the skin surface (Figure 8.10, Chapter 8). However, while the other tissue depths at greater than 2 cm and up to 5 cm only demonstrated an increase of about 1°C, the presence of bone in the tissue specimens could cause greater heating in tissues directly above the bone due to reflection of the ultrasonic energy by as much as three to four times. In most clinical applications, this is highly possible, and could possibly push the increase in temperatures at these deeper sites into the therapeutic range. The preliminary proposed model, therefore, should include data up to a depth of 5 cm. This would generally cover almost all the structures that can be considered as potential target tissues, and hence, can be considered reasonably comprehensive for most, if not all, clinical applications.

e. **Other considerations:**

1. In order to determine the depth of the target tissue as accurately as possible, a sound knowledge of anatomy, physiology and pathology is a pre-requisite.

2. In order to determine the correct amount of heating desired at the target tissue, a sound knowledge of the therapeutic effects which

can be achieved at the various therapeutic ranges is necessary. Some of this information is already available (Lehman and deLateur 1982, Kanui 1987) and can serve as preliminary guidelines. However, additional basic research (cellular and animal studies) may be necessary.

3. The size of the treatment area has been shown to significantly affect the amount of heating in the target issues (Chapter 6). For the most effective heating of tissues at deeper target sites (between 3 to 5 cm below surface), a treatment area corresponding to twice the size of the transducer is recommended based on the results of our study.

4. From the literature review, the ultrasonic gel should be applied at room temperature, and the transducer moved at a reasonable speed (recommended speed is 120 beats/min) in a to-and-fro direction.

5. As far as is possible, the transducer should be kept at a perpendicular angle to the tissues and sufficient pressure should be applied to maintain contact with the tissues at all times without causing deformation to the underlying tissues.

6. In order to minimize the error in the output intensity of the ultrasound, the ultrasound device used in research and clinical practice should be calibrated on a regular basis; i.e. at least once a month if used regularly, and once in six months if used infrequently.

Based on the above guidelines, the proposed model is presented in Table 9.7 and Figure 9.3. Essentially, Table 9.7 and Figure 9.3 can be used by the clinician or researcher as a reference guide for determining treatment

dosages at 1 MHz frequency, for a 10 minutes duration of exposure, at any target tissue site up to 5 cm. Prior to using the table or figure, the clinician or researcher has to determine the depth of the target tissue from the skin surface (in centimeters), and the desired increase in tissue temperature at the target site in order to achieve the required therapeutic effects. Table 9.7 or Figure 9.3 can then be referred to in order to select the required intensity that will achieve the desired amount of heating at the target tissue.

9.6 SUMMARY

- The test-retest reliability of the infrared spot thermometer was found to be slightly less reliable than the infrared video thermography unit, although both were deemed acceptable for the measurement of the main dependent variable in this series of experiments.
- An unplanned analysis of the twenty minutes of data (at one minute intervals) suggested the possibility of reducing the duration factor from 20 to 4 (5^{th}, 10^{th}, 15^{th} and 20^{th} minutes). In this manner, the data analyses for subsequent studies could be simplified considerably without affecting the overall results.
- Varying the movement speed of the transducer did not have an effect on the temperature increase within the tissues exposed to ultrasound, up to a depth of 5 cm, regardless of both the frequency of the transducer and duration of exposure / post-exposure. However, it is probably prudent to move the transducer quickly rather than slowly in order to minimize the sensation of "hot spots" for the subject, and the recommended speed for subsequent studies as well as for clinical practice was 120 beats/minute (or moderate speed).

Table 9.7: Means and Standard Deviations (SD) for increase in temperatures for 1 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 1.0 Watt/cm^2 after 10 minutes exposure

Intensity	Surface		1cm		2cm		3cm		4cm		5cm	
(W/cm^2)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.1	3.16	1.31	1.38	0.50	0.70	0.27	0.45	0.33	0.22	0.33	0.16	0.23
0.3	7.30	1.29	2.45	0.47	1.33	0.53	0.68	0.23	0.52	0.32	0.36	0.23
0.5	10.30	1.09	4.05	0.80	2.53	1.10	1.35	0.48	0.99	0.36	0.72	0.14
0.7	9.96	1.83	2.90	0.80	1.58	0.54	0.94	0.35	0.59	0.23	0.51	0.28
0.9	14.58	1.99	5.28	1.13	3.39	0.59	1.94	0.35	1.41	0.30	1.09	0.43
1.0	13.75	2.09	5.88	1.60	3.25	1.48	1.88	1.12	1.26	1.02	0.91	0.97
1.1	15.88	2.70	5.49	1.39	4.03	1.76	2.13	0.85	1.48	0.87	1.33	0.94
1.3	16.38	3.02	6.09	1.93	4.34	1.31	2.39	0.87	1.51	0.40	1.31	0.38
1.5	20.86	2.05	7.19	0.88	4.70	1.54	3.36	1.42	2.64	1.34	2.05	1.09

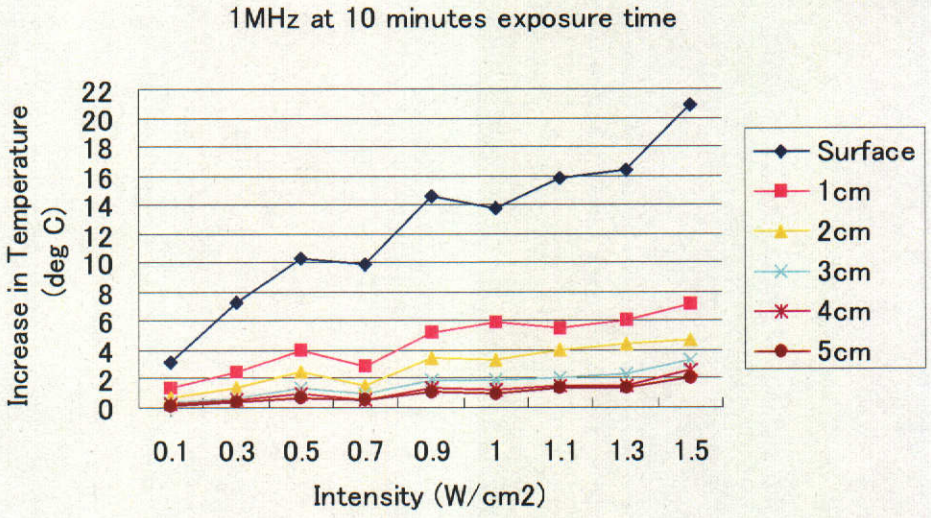

Figure 9.3: Proposed model for determining treatment dosages based on a 1MHz frequency device at 10 minutes exposure time.

- Smaller treatment sizes appeared to produce greater changes in temperatures at all tissue sites, compared with larger treatment sizes. The size of the treatment area, therefore, is an important consideration when clinicians or researchers attempt to estimate the amount of heating at the target tissue, or when attempting to formulate a dosage for treatment. Based on the results of our study, it was recommended that treatment sizes be standardized to 2X ERA to achieve the greatest depth of heating in the target tissues.
- Comparison of the results from our study with other similar studies performed on different types of tissue specimens suggested that the presence of bone in the tissue specimens could cause greater heating (by approximately three to four times) in tissues directly above the bone due to reflection of the ultrasonic energy.
- Based on the results of the qualitative histological studies, it was recommended that intensities up to 1.5 Watts/cm^2 (for both 1 and 3 MHz) be used for subsequent studies, as well as for clinical practice, as the safety of the patient, beyond these intensities, cannot be guaranteed.
- The results from the main study demonstrated that the increase in temperature due to absorption of the ultrasonic energy at any of the investigated target site (up to 5 cm below surface) was related to the ultrasound frequency, the output intensity and duration of the ultrasound exposure / post-exposure.
- The evidence for the frequency factor seems to suggest that compared with the 3 MHz, the 1 MHz frequency may be more appropriate for clinical applications as it does not overheat surface tissues, and at the same time, is able to increase the temperatures of target tissues up to a depth of 5 cm.
- If the aim of the therapeutic ultrasound application is to achieve thermal effects at the target tissues without causing overheating at the surface of the skin, the

results suggested that for 1 MHz frequency, the therapeutic range was between 0.5 to 1.3 Watts/cm^2 for target tissues up to 5 cm, and for 3 MHz frequency, the therapeutic range was between 0.3 to 0.5 Watts/cm^2 for target tissue up to 1 cm only. The minimum exposure time for both frequencies was 10 minutes. These results also suggested that the narrow therapeutic range for 3 MHz could render it questionable for clinical applications. The larger therapeutic range available for the 1 MHz frequency suggests that it is more suitable for clinical applications and research.

- The evidence for the duration factor seems to suggest that the recommended minimum exposure time would be 10 minutes in order to bring the temperature of the target tissues up to the therapeutic range, and to maintain this for at least 5 minutes as proposed by Lehman and deLateur (1982). For deeper tissues at target depths greater than 2 cm, the evidence from the heating pattern seems to suggest that increase in temperatures can be achieved in both the exposure and post-exposure phases. However, the effect of local circulation on this heating pattern is unknown and may counteract any additional heating the post-exposure phase.
- The evidence for the site of the target tissue factor seems to suggest that depths up to 2 cm below the surface is capable of reaching the therapeutic range easily. For depths greater than 2 cm and less than 5 cm, however, the presence of any bony structures within these tissues is a high possibility but is not necessarily a disadvantage as it could possibly increase the change in temperatures by three to four times, hence pushing the expected increase in temperature into therapeutic range.
- The results of our main study demonstrated that a higher frequency, a higher intensity, a greater exposure time and a more superficial site all contribute to a

greater change in temperature. A comparison of the results with other similar studies indicated that but for one exception (Draper et al 1995b), there were remarkable similarities (within mean±1SD) in the reported change in temperatures for the corresponding intensity and tissue site (indicated by bold fonts in Tables 9.1 and 9.2). Based on the limited comparisons between the results of our study and *in vivo* studies reported in the literature, the remarkable similarities could be an indication that the effect of circulation, which was absent from our study, appears to be within a variance of one standard deviation.

- A re-evaluation of 34 clinical studies reviewed in the literature survey (Chapter 2) was performed to assess the appropriateness of the dosage selected for investigation in these studies and its effect on the outcome of the study. When the studies with inadequate information regarding their treatment dosages were excluded, the percentage of true positives, true negatives, false positives and false negatives increased to 45%, 45%, 5% and 5% respectively. Hence, the accuracy of predicting a true positive or a true negative outcome by using the guidelines based on the results of this study was estimated to be about 90%. This would strongly suggest that a preliminary model to determine treatment dosages based on the results of this study was highly possible.
- The lack of a common model to determine treatment dosages could have been a significant factor affecting the results of clinical trials. There is a need to develop a common model for determining treatment dosages that can be used to guide both clinicians and researchers in the use of therapeutic ultrasound. Based on several specific guidelines developed from the results of our series of studies, a preliminary model was proposed and summarized in Table 9.7 and Figure 9.3. Essentially, the preliminary model can be used by the clinician or researcher as a reference for determining treatment dosages by selecting the

desired intensity which will produce the required amount of heating at the target tissue for a 1 MHz frequency, for a 10 minutes duration of exposure, at any target tissue site up to 5 cm.

CHAPTER TEN

CONCLUSION

The aim of this study was to clarify the relationship between the various dosage parameters used in therapeutic ultrasound, and to explore the possibility of proposing a preliminary model for determining treatment dosage in therapeutic ultrasound applications, both in the clinics for the treatment of patients, and in research for the conduct of clinical trials. The results of our investigations into the relationship of the various dosage parameters (frequency, intensity, duration of exposure and site of target tissue) were able to provide the evidence necessary for the development of a preliminary model, and this has been presented in Chapter 9. While the preliminary model is acknowledged as a first step effort only, and is far from complete, it can be refined further through immediate adoption and use by physical therapists and other users of therapeutic ultrasound. Through the feedback and experience gained from using such a model, the steps needed to finalise it will become clearer to future researchers. Only through a close collaboration between clinicians and researchers can a useful and accurate model be eventually developed.

10.1 CLINICAL RELEVANCE OF THE PROPOSED MODEL

The preliminary model was developed, not only to standardise the manner in which researchers determine their treatment dosages in clinical trials, but also with the clinician in mind. Because of this, the model was intentionally made as simple as possible and summarized in one table or figure so that it can be quickly accessed

and referred to by a busy clinician. In addition, only relevant parameters, based on the results of our study, were included in order to facilitate the clinical decision making process, rather than complicate it further. In this regard, the frequency parameter was reduced to just 1 MHz, and the duration of exposure was standardised at 10 minutes. In addition, the depth of target tissue covered an area up to 5 cm below the skin surface to accommodate for almost all of the possible target tissues that a clinician might consider treating with therapeutic ultrasound. The intensity range was also confined to the "therapeutic range", and high intensities that have been shown by our results to have the ability or potential to cause tissue damage were excluded from the model.

Comparisons made with other clinical studies (Chapter 9) suggested that the model is quite accurate in predicting true positive outcomes for the treatment of inflammatory conditions such as lateral epicondylitis and calcific tendonitis of the shoulder, among others. In addition, there also appeared to be some potential for the application of the model in the treatment of ulcers. The reason why these two conditions have demonstrated a potential for being successfully treated with therapeutic ultrasound could be related to the ease with which the ultrasonic energy was able to reach the target tissues, and subsequently, to increase the temperatures of the target tissues up to the therapeutic range. For lateral epicondylitis and calcific tendonitis, the target tissues are directly above the bony structures. Hence, even at low output intensities, the increase in tissue temperature at the target site is possible through reflection of the energy from the bone back into the tissues. An earlier comparison of our results with other studies (Chapter 9) seemed to indicate that reflection of the ultrasonic energy was capable of increasing the temperatures up to 3 or 4 times more. For the treatment of ulcers with therapeutic ultrasound, the target tissues are very superficial, and often exposed without the presence of skin or fat.

Again, these conditions were ideal for the ultrasonic energy to reach the target tissues easily and to cause an increase in tissue temperatures up to the therapeutic range even with very low intensities.

The above two examples illustrate an important finding that has been discussed in Chapter 9; that is, the amount of heating at the target site is directly related to the output intensity, and inversely related to the distance from the skin surface. When attempting to use the model for determining treatment dosages, this relationship can be used to guide the clinical decision making process, which can be divided into two categories: for target tissues up to 2 cm, and for target tissues between 2 and 5 cm below the skin surface.

a. **Target tissues sited between skin surface and 2 cm below**: The results of our study demonstrate that the target tissue temperatures between skin surface and 2 cm below can be easily increased up to the therapeutic range. For target tissues without the presence of bony structures, the middle range of intensities from 0.5 to 1.0 Watts/cm^2 would appear to be appropriate, such as in the treatment of superficial muscle injuries (eg. myosfascial pain and delayed onset muscle soreness), and neural structures that are embedded within these superficial muscles. For target tissues that are in close proximity to bony structures, the lower range of intensities from 0.1 to 0.5 Watts/cm^2 would appear to be appropriate, such as in the treatment of lateral epicondylitis, ligamentous ankle injuries, temporo-mandibular joint dysfunction, among others. These lower range of intensities (0.1 to 0.5 Watts/cm^2) would also appear to be appropriate for target tissues at the surface or very close to the surface, such as in the treatment of skin ulcers and episiotomy incisions, among others.

b. Target tissues sited between 2 and 5 cm below the skin surface: The

results of our study demonstrate that the target tissues temperatures between 2 and 5 cm below are not as easy to increase up to the therapeutic range. For target tissues without the presence of bony structures, the higher range of intensities from 1.0 to 1.5 Watts/cm^2 would appear to be appropriate, such as in the treatment of deep muscle injuries (eg. chronic muscle tears), and neural structures that are embedded within these deep muscles. For target tissues that are in close proximity to bony structures, the middle range of intensities from 0.5 to 1.0 Watts/cm^2 would appear to be appropriate, such as in the treatment of meniscal and cruciate ligament injuries in the knee joint, and arthritic conditions affecting the knee, shoulder or hip joints, among others.

Finally, the proposed preliminary model is for a small treatment area (2X ERA) because our results demonstrate that this was the optimum size for achieving the most effective heating of tissues subcutaneously. For target tissues that are large and cover an area greater than 2X ERA, it is advisable to divide the treatment area into multiples of 2X ERA and to regard each area separately if the composition of the tissues within each of these areas is considered significantly different (eg. in the treatment of the neck and back).

10.2 LIMITATIONS OF THE PROPOSED MODEL

The proposed model is only a preliminary attempt to provide clinicians and researchers with a common basis for determining treatment dosages based on desired amount of heating at the target tissues. The limitations of the proposed model are as follows:

a. Post-mortem porcine specimens used in this study did not have their local circulation and homeostatic mechanisms intact. Both these factors can

influence the amount of heating produced in the tissues by therapeutic ultrasound. The overall cooling effect of circulation on the heating pattern is about –1°C to –3°C (Lehmann et al 1966b). Comparisons between our data and similar studies using *in vivo* animal or human subjects demonstrate that it is possible to correct for the cooling effects of circulation on our present data by using the (mean – 1SD) values to account for the cooling effects, rather than using just the mean values. However, this correction factor may require further investigation prior to being included in the model.

b. The specimens used in this study were confined to skin, fat and muscle tissues without the presence of bone up to a depth of 5 cm. The presence of bone within the tissues can distort the transmission of ultrasound energy through the tissues, and hence, influence the heating pattern. This factor must be taken into account when clinicians or researchers use the preliminary model to determine treatment dosages for applications around bony areas.

c. The data obtained in the present study and used in the proposed model pertains to a specific ultrasound device (Omnisound 3000™, Physio Technology Inc., Topeka, Kansas, USA). While there is no reason to suspect that similar devices, with similar equipment specifications (e.g. ERA, BNR, etc.) and output intensities, would not produce the same type of heating pattern, the same cannot be assumed for ultrasound devices that differ markedly from the type used in this study.

10.3 FUTURE STUDIES

As a preliminary proposal, there are several limitations to the model that will require further investigation. These three limitations have already been

addressed; the cooling effect of circulation, the presence of bony structures in close proximity to the target tissues, and the generalizability of the results to different models of therapeutic ultrasound; and they should be the subject of further investigations in the future. In addition to these, the usefulness of refining the model to be based on one variable (total power), rather than two variables (intensity and duration), should be explored in future studies. Furthermore, it is also necessary to investigate how much ultrasonic energy (measured by temperature increase in the tissues) is needed to effect the various biophysical changes in the target tissues. It is hoped that subsequent studies will further clarify and improve upon the preliminary model, leading to a more robust and evidence-based model for determining treatment dosages in therapeutic ultrasound.

In conclusion, therapeutic ultrasound has been used by physical therapists and other professions for more than 5 decades with mixed success, in the treatment of various types of medical conditions. The biggest problem for any user is in determining the appropriate treatment dosages for the various types of conditions. Present data available from textbooks and journals do not provide enough information to facilitate the clinical decision making process for determining treatment dosages in therapeutic ultrasound which will ensure a successful outcome. The development of this preliminary model, based on guidelines formulated from experimental evidence regarding the relationship of the various dosage parameters, is only the first step towards addressing this problem. The success of this model would depend not only on future studies, by us and other researchers, to refine it further, but more importantly, on its acceptance by users of therapeutic ultrasound internationally. This acceptance by the users and the physical therapy profession in particular, is perhaps the biggest step towards the realization of an evidence-based model for determining treatment dosages for therapeutic ultrasound.

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Appendix 1

CORRELATION OF INFRARED SPOT THERMOMETER (MINOLTA HT-11) AND INFRARED VIDEO THERMOGRAPHY UNIT (AVIO TVS 2000) IN MEASUREMENT OF TEMPERATURE INCREASES IN POST-MORTEM PIG TISSUES EXPOSED TO THERAPEUTIC ULTRASOUND

1. INTRODUCTION

Infrared thermometry is non-invasive technique being used in current research to measure tissue and body temperatures (Armstrong et al 1997). There are two types of infrared thermometry commonly being used: the infrared spot (beam) thermometer and the infrared video thermography unit.

Infrared spot thermometer, such as the Minolta HT-11 (Minolta Co. Ltd., Japan) is a portable non-contact infrared thermo-sensor. It displays the temperature in degree Celsius, in increments of 0.1°C. The Minolta HT-11 was used in this series of experiments to measure the surface skin temperature in post-mortem pig tissues exposed to therapeutic ultrasound. The details of this instrument are described in Chapter 3 (p91).

The Avio TVS 2000 (Nippon Avionics Co. Ltd., Japan) infrared video thermography unit is a video thermal imaging system with radiometric temperature measurement capability. It has sensitivity up to 0.01°C and can provide both point and area measurement of temperatures. It was used in this series of experiments to measure the subcutaneous temperatures (at 1, 2, 3, 4 and 5 cm below skin surface) in post-mortem pig tissues exposed to therapeutic ultrasound. The details of this instrument are described in Chapter 3 (pp89-90).

The validity of infra-red video thermography, Inframetrics 600M (Inframetrics Inc., Bedford, Massachusetts, USA) and the infra-red spot thermometer, FirstTemp 2000A (Intelligent Medical Systems Inc., Carlsbad, California, USA) were investigated by Sherman et al (1996). Comparisons were made between the two recording instruments using a heat-producing reference device. Sherman et al (1996) reported a Spearman's correlation coefficient of 0.994 for the infra-red video thermography and 0.990 for the infra-red spot (beam) thermography. They concluded that both infra-red video (Inframetrics 600M) and spot thermometer (FirstTemp 2000A) devices were highly valid and accurate instruments for measurement of temperatures.

There have been no studies that examined the correlation between the Avio TVS 2000 and the Minolta HT-11. The aim of this study was to correlate the Avio TVS 2000 and the Minolta HT-11 in the measurement of temperature increases in post-mortem pig tissues exposed to therapeutic ultrasound.

2. METHODS

a. Specimens

Three tissue specimens, obtained from adult post-mortem pigs, were processed as previously described (Chapter 3, Section 3.4, pp92-94).

b. Instrumentation and Procedure

Therapeutic ultrasound at 1 MHz was applied to the tissues at 1.0 Watt/cm^2 for 10 minutes. The size of the treatment area was standardized at twice the size of the transducer, and was maintained using a thermoplastic guide, secured over the shaved skin of the designated treatment area. A direct, in-contact, moving soundhead technique using ultrasonic gel was applied. The movement speed of the

transducer was standardized at 120 beats/minute, maintained using a metronome.

The Minolta HT-11 and the Avio TVS 2000 were used to measure the temperature of the heated tissues at the same spot. Emissivity was set at 0.98 for both devices to correspond to the emissivity for human tissues. Duplicate measurements, one with each device, were repeated 15 times on each of the three specimens as the tissue temperature changed with increased exposure.

At the end of the experiments, the specimens were disposed of according to the procedure described in Chapter 3 (section 3.4, paragraph j, p100)

c. Data Analysis

Data were analysed using the SPSS for Windows software, Version 10.0 (SPSS Inc., 444N Michigan Avenue, Chicago, Illinois 60611, USA). An intraclass correlation coefficient (single measure, two-way mixed effect) was used to estimate the absolute agreement between the two measuring devices. The confidence interval was set at 95%.

3. RESULTS

A summary of the temperature measurements (raw data) for the three specimens is given in Table A1-1.

The results show a correlation coefficient of 0.99 ($F_{44, 44}$=218.21; $p<0.01$) for the two measuring devices, indicating an excellent agreement between the temperatures recorded by both devices.

Table A1-1: Summary of temperature measurements (°C) for the three specimens.

Specimen 1			Specimen 2			Specimen 3		
Sno	HT-11	TVS 2000	Sno	HT-11	TVS 2000	Sno	HT-11	TV S2000
1	13.4	13.6	16	12.0	12.4	31	17.9	18.1
2	9.4	8.9	17	15.9	15.4	32	23.1	22.4
3	13.7	13.8	18	13.7	13.4	33	22.5	21.9
4	9.5	8.9	19	16.1	16.1	34	21.1	20.4
5	14.0	13.6	20	16.2	16.9	35	18.3	18.3
6	13.9	13.8	21	16.6	16.9	36	16.9	16.7
7	10.1	9.8	22	14.0	14.5	37	22.2	21.2
8	15.0	15.8	23	17.8	17.5	38	21.9	21.0
9	12.0	12.3	24	15.8	15.5	39	20.6	20.0
10	14.9	14.9	25	18.2	17.8	40	19.2	19.1
11	11.9	12.4	26	16.4	15.7	41	24.1	24.0
12	7.4	8.6	27	18.2	17.7	42	24.2	23.2
13	12.0	12.9	28	17.2	16.4	43	23.5	23.2
14	15.0	15.9	29	17.8	18.0	44	21.6	21.6
15	15.1	15.9	30	17.0	16.9	45	20.1	20.4

4. DISCUSSION

The results of our study were consistent with those reported by Sherman et al (1996). The excellent agreement between the two measuring devices indicated that it was possible to use the Minolta HT-11 and the Avio TVS 2000 interchangeably in our series of experiments.

5. CONCLUSION

There was excellent agreement between the Minolta HT-11 and the Avio TVS 2000 in the measurement of temperature increases in post-mortem pig tissues exposed to therapeutic ultrasound.

CALIBRATION PROCEDURE FOR THE OMNISOUND 3000

The calibration procedure is entirely software driven by the Omnisound 3000^{TM} ultrasound machine. The setup of the power meter and ultrasound machine is given in Section 3.3.4a (Chapter 3). Following the setup, the calibration procedure is activated from the main menu on the display panel of the ultrasound machine (see Screen 1 below). Once this has been activated, it remains only to follow the step-by-step instructions given on the display panel of the ultrasound machine. The following is a reproduction of the panel displays in sequential order, and is self-explanatory (additional explanations are given in italics).

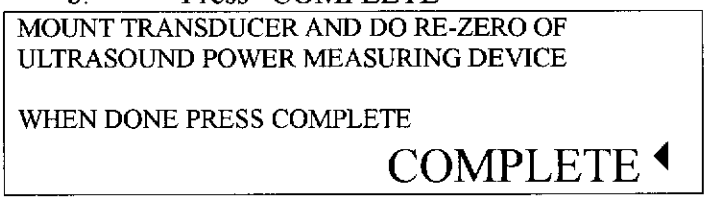
1. Screen 1: Initial Display
   a. Select "CALIBRATION"

0.0 W/CM^2	0 JOULES
TIME MIN : SEC	SELECT DISPLAY W OR W/CM^2 ◀
▲ 5 : 00 ▼	◀
DUTY FACTOR	TEMP MODE SET UP ◀
CONT	
100 %	CALIBRATION ◀
FREQUENCY	
DEEP	TRANSDUCER
1 MHZ ☐)))))))	BNR : 1.3
	ERA : 1.5 CM^2

2. Screen 2 : Calibration (Start Up)
   a. Carry out instructions on screen
   b. Press "CONTINUE"

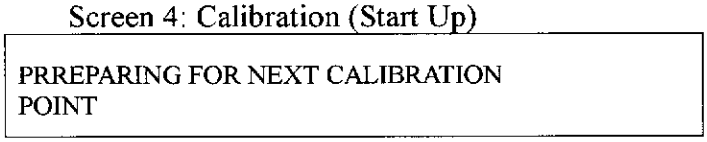
CALIBRATION	REQUIRED INSTRUMENTS
• CALIBRATION REQUIRES THE USE OF OHMIC UPM 10 /30 OR EQUIVALENT ULTRASOUND BALANCE • USE DEGASSED WATER LESS THAN 4 PPM DISSOLVED OXYGEN. USE O-12 CHEMETRICS INC. OR EQUIVALENT TO VERIFY DISSOLVED OXYGEN LEVEL • SET WATER TEMP TO 22 C +/- 3 C • LEVEL WATTMETER AND CHECK FOR BUBBLES	
PRESS CONTINUE OR RETURN	
▶ CONTINUE	RETURN ◀

3. Screen 3 : Calibration (Start Up)
   a. Carry out instructions on screen.
   b. Press "COMPLETE"

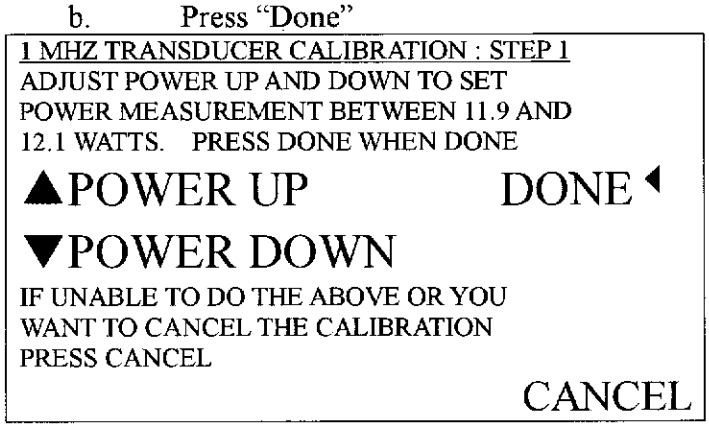


Since the instruments have already been setup prior to activating the calibration program, just press "COMPLETE" *and proceed with the next step.*

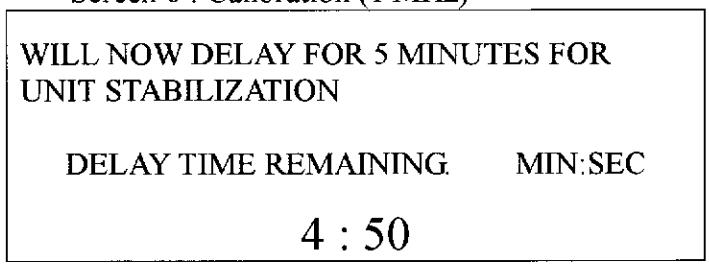
4. Screen 4: Calibration (Start Up)



5. Screen 5: Calibration (1 MHz)
   a. Select "Power Up" or "Power Down" as indicated
   b. Press "Done"



6. Screen 6 : Calibration (1 MHz)



The countdown from 5 minutes will begin. At this stage, it is critical neither to touch any of the instruments setup nor to shake the table. A completely stable environment is necessary to ensure minimum artifacts. After 5 minutes, the screen display will change automatically to the next screen.

7. Screen 7 : Calibration (1 MHz)
   a. Select "Power Up" or "Power Down" as indicated
   b. Press "Done"

1 MHZ TRANSDUCER CALIBRATION : STEP 2
ADJUST POWER UP AND DOWN TO SET
POWER MEASUREMENT BETWEEN 11.9 AND
12.1 WATTS. PRESS DONE WHEN DONE
▲POWER UP DONE
▼POWER DOWN
IF UNABLE TO DO THE ABOVE OR YOU WANT
TO CANCEL THE CALIBRATION PRESS CANCEL
CANCEL

8. Screen 8 : Calibration (1 MHz)

PRREPARING FOR NEXT CALIBRATION
POINT

9. Screen 9 : Calibration (1 MHz)
   a. Select "Power Up" or "Power Down" as indicated
   b. Press "Done"

1 MHZ TRANSDUCER CALIBRATION : STEP 3
ADJUST POWER UP AND DOWN TO SET
POWER MEASUREMENT BETWEEN 8.9 AND
9.1 WATTS. PRESS DONE WHEN DONE
▲POWER UP DONE
▼POWER DOWN
IF UNABLE TO DO THE ABOVE OR YOU WANT
TO CANCEL THE CALIBRATION PRESS CANCEL
CANCEL

10. Screen 10: Calibration (1 MHz)

PRREPARING FOR NEXT CALIBRATION
POINT

11. Screen 11: Calibration (1 MHz)
   a. Select "Power Up" or "Power Down" as indicated.
   b. Press "Done"

1 MHZ TRANSDUCER CALIBRATION : STEP 4
ADJUST POWER UP AND DOWN TO SET
POWER MEASUREMENT BETWEEN 4.9 AND
5.1 WATTS. PRESS DONE WHEN DONE
▲POWER UP DONE
▼POWER DOWN
IF UNABLE TO DO THE ABOVE OR YOU WANT
TO CANCEL THE CALIBRATION PRESS CANCEL
CANCEL

12. Screen 12: Calibration (1 MHz)

PRREPARING FOR NEXT CALIBRATION
POINT

13. Screen 13: Calibration (1 MHz)
    a. Select "Power Up" or "Power Down" as indicated.
    b. Press "Done"

1 MHZ TRANSDUCER CALIBRATION : STEP 5
ADJUST POWER UP AND DOWN TO SET
POWER MEASUREMENT BETWEEN 1.9 AND
2.1 WATTS. PRESS DONE WHEN DONE

▲POWER UP DONE ◀

▼POWER DOWN

IF UNABLE TO DO THE ABOVE OR YOU
WANT TO CANCEL THE CALIBRATION
PRESS CANCEL

CANCEL

14. Screen 14: Calibration (1 MHz)

PRREPARING FOR NEXT CALIBRATION
POINT

This marks the end of calibration for 1MHz. Following on from this is the calibration procedure for 3 MHz. These two procedures are uninterrupted.

15. Screen 15: Calibration (3 MHz)
    a. Select "Power Up" or "Power Down" as indicated.
    b. Press "Done"

3 MHZ TRANSDUCER CALIBRATION : STEP 1
ADJUST POWER UP AND DOWN TO SET
POWER MEASUREMENT BETWEEN 8.9 AND
9.1 WATTS. PRESS DONE WHEN DONE

▲POWER UP DONE ◀

▼POWER DOWN

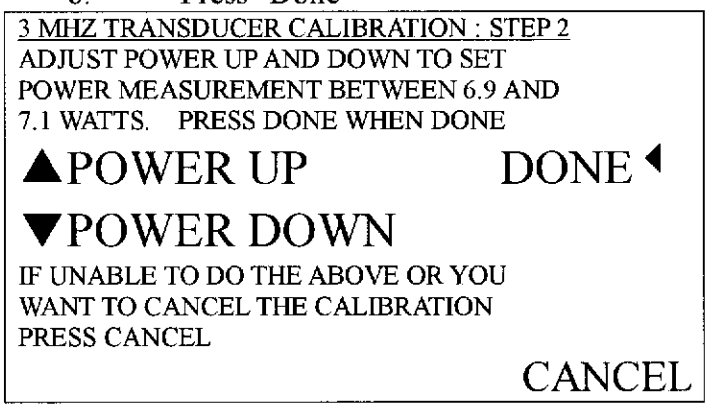
IF UNABLE TO DO THE ABOVE OR YOU
WANT TO CANCEL THE CALIBRATION
PRESS CANCEL

CANCEL

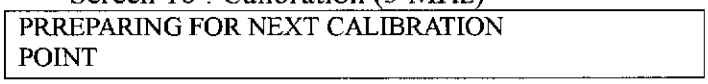
16. Screen 16 : Calibration (3 MHz)

PRREPARING FOR NEXT CALIBRATION
POINT

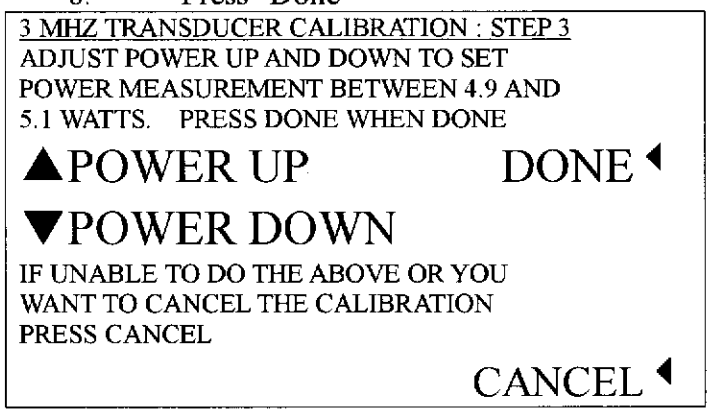
17. Screen 17: Calibration (3 MHz)
    a. Select "Power Up" or "Power Down" as indicated.
    b. Press "Done"



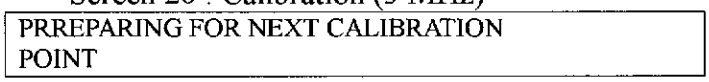
18. Screen 18 : Calibration (3 MHz)



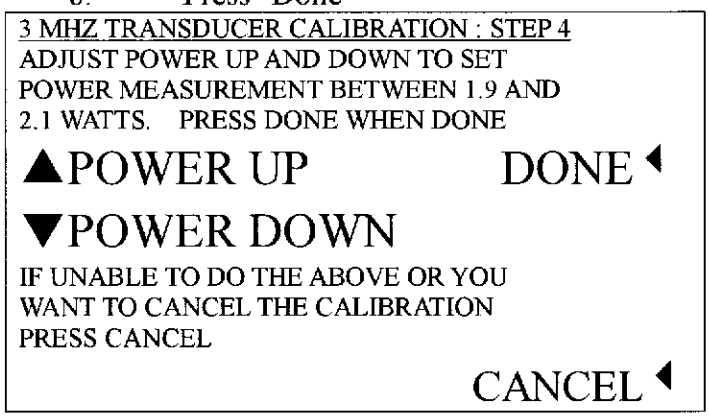
19. Screen 19 : Calibration (3MHz)
    a. Select "Power Up" or "Power Down"
    b. Press "Done"



20. Screen 20 : Calibration (3 MHz)



21. Screen 21 : Calibration (3MHz)
    a. Select "Power Up" or "Power Down"
    b. Press "Done"



22. Screen 22 : Calibration (3 MHz)

PRREPARING FOR NEXT CALIBRATION POINT

This essentially marks the end of the calibration procedure for both 1 MHz and 3 MHz. The next few screens are for housekeeping purposes and should be followed through to the end.

23. Screen 23 : Updating Year/Month/Day
    a. Select "Up" or "Down" button to set the correct month
    b. Press "Enter"

WILL NOW ENTER CALIBRATION DATE PRESS UP OR DOWN UNTIL CORRECT MONTH APPEARS BELOW ▲UP 11 ▼DOWN ENTER◄ PRESS ENTER WHEN CORRECT

24. Screen 24: Updating Year/Month/Day
    a. Select "Up" or "Down" button to set the correct day
    b. Press "Enter"

WILL NOW ENTER CALIBRATION DATE PRESS UP OR DOWN UNTIL CORRECT DAY APPEARS BELOW ▲UP 9 ▼DOWN ENTER◄ PRESS ENTER WHEN CORRECT

25. Screen 25 : Updating Year/Month/Day
    a. Select "Up" or "Down" button to set the correct year
    b. Press "Enter"

WILL NOW ENTER CALIBRATION DATE PRESS UP OR DOWN UNTIL CORRECT YEAR APPEARS BELOW ▲UP 98 ▼DOWN ENTER◄ PRESS ENTER WHEN CORRECT

26. Screen 26: Calibration: End

CALIBRATION COMPLETE

27. Screen 27: Machine re-starts
28. End of Calibration Procedure

TABLES OF MEANS AND INTRACLASS CORRELATION COEFFICIENTS FOR CHAPTER FOUR

Table A3-1: Means, standard deviations (SD), single measure intraclass correlation coefficients (ICCs) (two-way mixed effect model), 95% confidence intervals (CIs), F values, degrees of freedom (df), significance (p value) for infrared spot thermometer for 1MHz ultrasound (1.0Watt/cm^2, 2X ERA, 120beats/min) at skin's surface.

	Test		Retest			95% CI				
Time	Mean	SD	Mean	SD	ICC	Lower	Upper	F	df	Sig
1	5.68	0.77	5.29	0.79	0.34	-0.23	0.74	2.039	12	0.12
2	7.09	1.09	6.81	0.96	0.55	0.02	0.84	3.400	12	0.02
3	8.48	1.22	8.02	1.24	0.43	-0.14	0.78	2.496	12	0.06
4	9.68	1.66	9.00	1.22	0.49	-0.05	0.81	2.952	12	0.04
5	10.59	1.47	9.85	1.35	0.55	0.03	0.84	3.489	12	0.02
6	11.29	1.42	10.66	1.49	0.58	0.07	0.85	3.736	12	0.02
7	12.27	1.80	11.38	1.39	0.62	0.13	0.87	4.249	12	0.01
8	12.72	1.83	11.98	1.52	0.65	0.18	0.88	4.695	12	0.01
9	13.30	2.09	12.45	1.61	0.65	0.18	0.88	4.694	12	0.01
10	13.72	2.25	12.87	1.68	0.65	0.18	0.88	4.740	12	0.01
11	10.95	1.80	10.32	1.48	0.69	0.25	0.89	5.412	12	<0.01
12	9.55	1.72	9.03	1.42	0.55	0.03	0.84	3.488	12	0.02
13	8.67	1.76	8.13	1.38	0.58	0.07	0.85	3.731	12	0.02
14	7.68	1.69	7.52	1.42	0.67	0.21	0.89	5.003	12	<0.01
15	7.12	1.64	7.01	1.58	0.67	0.22	0.89	5.072	12	<0.01
16	6.62	1.69	6.43	1.46	0.66	0.20	0.88	4.945	12	<0.01
17	6.12	1.69	5.88	1.47	0.63	0.14	0.87	4.333	12	0.01
18	5.72	1.35	5.36	1.38	0.73	0.32	0.91	6.396	12	<0.01
19	5.36	1.43	5.12	1.45	0.61	0.11	0.86	4.083	12	0.01
20	5.05	1.50	4.74	1.30	0.61	0.12	0.86	4.182	12	0.01

Table A3-2: Means, standard deviations (SD), single measure intraclass correlation coefficients (ICCs) (two-way mixed effect model), 95% confidence intervals (CIs), F values, degrees of freedom (df), significance (p value) for infrared video thermography unit (Avio TVS 2000) for 1MHz ultrasound (1.0Watt/cm^2, 2X ERA, 120beats/min) at 1 cm below skin surface.

	Test		Retest			95% CI				
Time	Mean	SD	Mean	SD	ICC	Lower	Upper	F	df	Sig
1	0.94	0.48	1.19	0.78	0.51	-0.03	0.82	3.112	12	0.03
2	1.68	0.86	1.76	0.87	0.79	0.45	0.93	8.597	12	<0.01
3	2.54	1.27	2.48	1.10	0.53	<0.01	0.83	3.287	12	0.02
4	3.21	1.35	3.13	1.28	0.53	-0.01	0.83	3.238	12	0.03
5	3.80	1.56	3.56	1.26	0.57	0.05	0.84	3.614	12	0.02
6	4.21	1.62	4.02	1.35	0.57	0.05	0.85	3.635	12	0.02
7	4.70	1.72	4.41	1.23	0.34	-0.23	0.74	2.038	12	0.12
8	5.12	1.81	4.74	1.26	0.48	-0.07	0.81	2.826	12	0.04
9	5.53	1.66	4.98	1.32	0.57	0.05	0.84	3.618	12	0.02
10	5.83	1.71	5.48	1.40	0.65	0.18	0.88	4.663	12	0.01
11	5.07	1.67	4.89	1.34	0.76	0.37	0.92	7.182	12	<0.01
12	5.00	1.46	4.69	1.24	0.76	0.39	0.92	7.506	12	<0.01
13	4.79	1.39	4.36	0.87	0.67	0.22	0.89	5.093	12	<0.01
14	4.63	1.29	4.18	0.84	0.64	0.17	0.88	4.591	12	0.01
15	4.56	1.24	4.03	0.82	0.66	0.19	0.88	4.838	12	0.01
16	4.37	1.18	3.96	0.70	0.57	0.06	0.85	3.656	12	0.02
17	4.25	1.17	3.75	0.75	0.64	0.17	0.88	4.628	12	0.01
18	4.15	1.19	3.65	0.71	0.62	0.13	0.87	4.295	12	0.01
19	3.95	1.11	3.42	0.67	0.60	0.10	0.86	39.89	12	0.01
20	3.79	1.16	3.28	0.64	0.65	0.19	0.88	4.768	12	0.01

Table A3-3: Means, standard deviations (SD), single measure intraclass correlation coefficients (ICCs) (two-way mixed effect model), 95% confidence intervals (CIs), F values, degrees of freedom (df), significance (p value) for infrared video thermography unit (Avio TVS 2000) for 1MHz ultrasound (1.0Watt/cm^2, 2X ERA, 120beats/min) at 2 cm below skin surface.

	Test		Retest			95% CI				
Time	Mean	SD	Mean	SD	ICC	Lower	Upper	F	df	Sig
1	0.51	0.41	0.38	0.29	0.42	-0.15	0.78	2.445	12	0.07
2	0.72	0.60	0.62	0.41	0.44	-0.12	0.79	2.579	12	0.06
3	1.02	0.79	0.97	0.55	0.45	-0.11	0.79	2.647	12	0.05
4	1.40	0.79	1.23	0.69	0.58	0.07	0.85	3.786	12	0.01
5	1.75	1.06	1.61	0.84	0.57	0.05	0.85	3.643	12	0.02
6	2.08	1.22	1.92	0.88	0.57	0.05	0.85	3.635	12	0.02
7	2.37	1.34	2.30	0.99	0.56	0.03	0.84	3.507	12	0.02
8	2.58	1.26	2.60	1.00	0.57	0.06	0.85	3.658	12	0.02
9	2.96	1.43	2.80	1.03	0.59	0.08	0.85	3.855	12	0.01
10	3.27	1.58	3.14	1.25	0.75	0.92	0.92	6.845	12	<0.01
11	2.91	1.29	2.87	1.14	0.82	0.94	0.94	10.079	12	<0.01
12	3.03	1.16	3.00	1.15	0.78	0.93	0.93	8.154	12	<0.01
13	3.03	1.10	3.05	1.07	0.80	0.94	0.94	9.214	12	<0.01
14	3.09	1.11	3.00	1.06	0.75	0.92	0.92	6.987	12	<0.01
15	3.08	1.05	3.10	1.08	0.74	0.91	0.91	6.586	12	<0.01
16	3.05	1.00	3.14	1.06	0.78	0.93	0.93	8.102	12	<0.01
17	3.07	1.07	3.12	1.08	0.77	0.92	0.92	7.585	12	<0.01
18	3.14	1.10	3.10	1.07	0.75	0.92	0.92	6.967	12	<0.01
19	3.04	0.98	3.06	1.12	0.79	0.93	0.93	8.421	12	<0.01
20	2.98	1.01	2.91	1.07	0.81	0.94	0.94	9.649	12	<0.01

Table A3-4: Means, standard deviations (SD), single measure intraclass correlation coefficients (ICCs) (two-way mixed effect model), 95% confidence intervals (CIs), F values, degrees of freedom (df), significance (p value) for infrared video thermography unit (Avio TVS 2000) for 1MHz ultrasound (1.0Watt/cm^2, 2X ERA, 120beats/min) at 3 cm below skin surface.

	Test		Retest			95% CI				
Time	Mean	SD	Mean	SD	ICC	Lower	Upper	F	df	Sig
1	0.24	0.46	0.23	0.21	0.22	-0.35	0.68	1.580	12	0.22
2	0.27	0.45	0.27	0.25	0.51	-0.04	0.82	3.051	12	0.03
3	0.43	0.47	0.46	0.31	0.68	0.24	0.89	5.306	12	<0.01
4	0.68	0.53	0.69	0.42	0.80	0.47	0.93	8.982	12	<0.01
5	0.81	0.76	0.86	0.54	0.80	0.46	0.93	8.937	12	<0.01
6	1.05	0.80	1.03	0.58	0.91	0.73	0.97	21.089	12	<0.01
7	1.22	0.93	1.23	0.69	0.84	0.56	0.95	11.525	12	<0.01
8	1.43	0.96	1.43	0.76	0.90	0.70	0.97	18.583	12	<0.01
9	1.64	1.09	1.55	0.86	0.89	0.69	0.97	17.704	12	<0.01
10	1.87	1.20	1.85	0.96	0.92	0.75	0.97	23.115	12	<0.01
11	1.80	1.15	1.76	0.90	0.92	0.76	0.98	23.668	12	<0.01
12	1.87	1.12	1.84	0.92	0.90	0.72	0.97	19.678	12	<0.01
13	1.94	1.19	1.95	0.92	0.88	0.65	0.96	15.303	12	<0.01
14	1.95	1.08	1.95	0.94	0.93	0.78	0.98	27.104	12	<0.01
15	2.04	1.06	2.07	0.84	0.89	0.68	0.97	16.898	12	<0.01
16	2.07	0.96	2.09	0.85	0.90	0.72	0.97	19.843	12	<0.01
17	2.04	1.02	2.12	0.84	0.81	0.49	0.94	9.524	12	<0.01
18	2.12	0.98	2.06	0.80	0.85	0.58	0.95	12.440	12	<0.01
19	2.08	0.95	2.07	0.78	0.87	0.64	0.96	14.878	12	<0.01
20	2.13	0.89	2.02	0.78	0.91	0.74	0.97	22.018	12	<0.01

Table A3-5: Means, standard deviations (SD), single measure intraclass correlation coefficients (ICCs) (two-way mixed effect model), 95% confidence intervals (CIs), F values, degrees of freedom (df), significance (p value) for infrared video thermography unit (Avio TVS 2000) for 1MHz ultrasound (1.0Watt/cm^2, 2X ERA, 120beats/min) at 4 cm below skin surface.

	Test		Retest			95% CI				
Time	Mean	SD	Mean	SD	ICC	Lower	Upper	F	df	Sig
1	0.18	0.32	0.28	0.19	0.16	-0.41	0.64	1.385	12	0.29
2	0.23	0.34	0.23	0.28	0.66	0.20	0.88	4.899	12	0.01
3	0.33	0.43	0.41	0.30	0.58	0.07	0.85	3.784	12	0.01
4	0.44	0.47	0.46	0.39	0.82	0.52	0.94	10.411	12	<0.01
5	0.56	0.65	0.59	0.51	0.73	0.33	0.91	6.443	12	<0.01
6	0.68	0.72	0.72	0.59	0.83	0.52	0.94	10.497	12	<0.01
7	0.85	0.74	0.84	0.61	0.79	0.45	0.93	8.617	12	<0.01
8	0.98	0.83	0.99	0.71	0.87	0.63	0.96	14.434	12	<0.01
9	1.12	0.95	1.13	0.83	0.88	0.65	0.96	15.297	12	<0.01
10	1.26	1.07	1.30	0.87	0.89	0.68	0.97	16.862	12	<0.01
11	1.27	1.03	1.35	0.92	0.94	0.82	0.98	33.521	12	<0.01
12	1.36	1.00	1.41	0.93	0.94	0.83	0.98	34.452	12	<0.01
13	1.34	1.03	1.40	0.99	0.92	0.77	0.98	25.152	12	<0.01
14	1.38	0.94	1.50	0.89	0.92	0.75	0.97	23.130	12	<0.01
15	1.39	0.92	1.52	0.89	0.93	0.79	0.98	26.165	12	<0.01
16	1.48	0.95	1.52	0.92	0.89	0.67	0.96	16.627	12	<0.01
17	1.49	0.97	1.65	0.85	0.88	0.66	0.96	16.129	12	<0.01
18	1.53	0.99	1.60	0.88	0.91	0.73	0.97	20.851	12	<0.01
19	1.58	0.90	1.53	0.88	0.87	0.64	0.96	14.791	12	<0.01
20	1.53	0.90	1.54	0.84	0.89	0.67	0.96	16.782	12	<0.01

Table A3-6: Means, standard deviations (SD), single measure intraclass correlation coefficients (ICCs) (two-way mixed effect model), 95% confidence intervals (CIs), F values, degrees of freedom (df), significance (p value) for infrared video thermography unit (Avio TVS 2000) for 1MHz ultrasound (1.0Watt/cm^2, 2X ERA, 120beats/min) at 5 cm below skin surface.

	Test		Retest			95% CI				
Time	Mean	SD	Mean	SD	ICC	Lower	Upper	F	df	Sig
1	0.03	0.27	0.07	0.22	0.06	-0.49	0.57	1.129	12	0.42
2	0.05	0.37	0.12	0.36	0.51	-0.03	0.82	3.117	12	0.03
3	0.17	0.41	0.24	0.38	0.60	0.10	0.86	4.004	12	0.02
4	0.27	0.41	0.33	0.56	0.71	0.29	0.90	6.000	12	<0.01
5	0.33	0.63	0.43	0.71	0.80	0.47	0.94	9.019	12	<0.01
6	0.47	0.66	0.53	0.61	0.88	0.66	0.96	15.793	12	<0.01
7	0.62	0.77	0.67	0.76	0.85	0.58	0.95	12.334	12	<0.01
8	0.63	0.78	0.77	0.80	0.86	0.61	0.96	13.386	12	<0.01
9	0.78	0.87	0.87	0.99	0.89	0.68	0.97	17.218	12	<0.01
10	0.90	1.01	1.02	1.05	0.89	0.67	0.96	16.630	12	<0.01
11	0.90	1.03	1.05	1.09	0.91	0.72	0.97	20.094	12	<0.01
12	0.96	1.03	1.08	1.13	0.92	0.76	0.97	23.461	12	<0.01
13	0.95	0.99	1.13	1.15	0.89	0.68	0.97	17.129	12	<0.01
14	0.98	0.94	1.13	1.06	0.93	0.79	0.98	28.009	12	<0.01
15	1.04	0.88	1.19	1.02	0.88	0.66	0.96	15.873	12	<0.01
16	1.08	0.91	1.19	1.03	0.87	0.64	0.96	14.711	12	<0.01
17	1.00	0.91	1.18	1.02	0.93	0.80	0.98	29.252	12	<0.01
18	1.16	0.84	1.14	1.03	0.90	0.71	0.97	19.472	12	<0.01
19	1.06	0.85	1.14	0.97	0.90	0.71	0.97	19.604	12	<0.01
20	1.05	0.80	1.13	0.96	0.86	0.61	0.96	13.717	12	<0.01

Table A3-7: Intraclass correlation coefficients (ICCs) (single measure, 2-way mixed effect model), standard error of measurement (SEM) and 95% Confidence Interval (CI) for Infrared Spot Thermometer (Minolta HT11) (surface) and Infrared Video Thermography unit (Avio TVS 2000) (1, 2, 3, 4, and 5 cm below skin surface)

Site	Time (minutes)	ICC	Significance *p*	SEM	95% CI Lower	95% CI Upper
Surface	5	0.55	0.02	0.982	8.67	12.52
	10	0.65	0.01	1.328	10.57	15.77
	15	0.67	<0.01	0.587	5.97	8.27
	20	0.61	0.01	0.931	3.22	6.87
1 cm	5	0.57	0.02	1.026	1.79	5.81
	10	0.65	0.01	1.016	3.84	7.82
	15	0.66	0.01	0.724	3.14	5.98
	20	0.65	0.01	0.684	2.45	5.13
2 cm	5	0.57	0.02	0.695	0.39	3.11
	10	0.75	<0.01	0.796	1.71	4.83
	15	0.74	<0.01	0.542	2.02	4.15
	20	0.81	<0.01	0.437	2.12	3.83
3 cm	5	0.80	<0.01	0.340	0.14	1.47
	10	0.92	<0.01	0.346	1.20	2.55
	15	0.89	<0.01	0.355	1.34	2.73
	20	0.91	<0.01	0.264	1.61	2.65
4 cm	5	0.73	<0.01	0.335	-0.10	1.22
	10	0.89	<0.01	0.357	0.56	1.96
	15	0.93	<0.01	0.241	0.92	1.87
	20	0.89	<0.01	0.302	0.94	2.12
5 cm	5	0.80	<0.01	0.283	-0.22	0.89
	10	0.89	<0.01	0.339	0.23	1.56
	15	0.88	<0.01	0.302	0.45	1.63
	20	0.86	<0.01	0.296	0.47	1.63

Appendix 4

TABLES OF MEANS AND THERMOGRAPHIC SCANS FOR CHAPTER FIVE

Table A4-1: Means and standard deviations (SD) for 1 MHz and 3 MHz ultrasound (1.0Watt/cm^2, 2X ERA) at slow (60 beats/min), moderate (120 beats/min) and fast (180 beats/min) movement speed of transducer at skin's surface.

Time	1 MHz						3 MHz					
	Slow		Moderate		Fast		Slow		Moderate		Fast	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	6.30	1.43	5.49	0.51	6.16	1.53	10.20	1.99	10.39	1.50	10.76	1.39
2	8.39	1.69	7.20	0.48	8.26	1.90	14.73	2.70	14.49	1.17	14.91	1.69
3	9.89	2.08	8.76	0.89	9.26	1.95	17.72	2.67	17.45	1.44	17.39	1.95
4	10.86	1.89	10.03	1.35	10.64	2.21	19.92	2.23	19.26	1.68	19.84	2.06
5	11.86	1.85	10.80	1.63	11.51	2.41	21.98	2.40	21.16	1.50	21.48	2.32
6	12.75	2.02	11.88	1.73	12.23	2.45	23.23	2.66	22.83	1.40	23.02	2.24
7	13.65	1.92	12.53	1.81	12.95	2.61	24.39	2.79	23.44	1.69	23.83	2.36
8	14.20	1.86	13.03	1.70	13.67	2.69	25.22	2.46	24.61	1.70	24.83	2.41
9	15.03	2.30	13.61	1.85	14.21	2.55	26.21	2.79	25.51	1.96	25.82	2.56
10	15.16	2.04	14.18	1.92	14.74	2.86	27.11	2.83	26.22	1.76	26.05	2.56
11	12.94	2.23	11.61	2.18	11.77	2.62	20.93	3.23	20.74	1.66	20.55	2.45
12	10.83	1.95	9.92	2.38	9.99	2.60	18.06	3.06	17.88	2.08	17.36	2.40
13	9.72	1.79	9.04	2.30	9.12	2.49	16.16	2.59	15.76	1.84	15.59	2.28
14	8.66	1.56	8.17	1.99	8.31	2.49	14.68	2.56	14.23	1.81	13.93	2.11
15	7.93	1.76	7.31	2.19	7.49	2.42	13.39	2.39	13.07	1.66	12.81	1.94
16	7.27	1.60	6.64	2.01	6.83	2.43	12.26	2.30	12.13	1.67	11.89	1.92
17	6.77	1.65	6.20	2.05	6.26	2.39	11.47	2.34	11.26	1.47	10.86	1.75
18	6.29	1.72	5.75	2.02	5.71	2.34	10.60	2.41	10.33	1.51	10.13	1.76
19	5.89	1.65	5.47	1.95	5.42	2.33	9.84	2.13	9.65	1.61	9.40	1.80
20	5.45	1.55	5.01	1.90	5.12	2.25	9.30	2.26	9.08	1.70	8.79	1.73

Table A4-2: Means and standard deviations (SD) for 1 MHz and 3 MHz ultrasound (1.0Watt/cm^2, 2X ERA) at slow (60 beats/min), moderate (120 beats/min) and fast (180 beats/min) movement speed of transducer at 1 cm below the skin's surface.

Time	1 MHz						3 MHz					
	Slow		Moderate		Fast		Slow		Moderate		Fast	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.67	0.42	0.69	0.40	0.49	0.27	0.52	0.49	0.55	0.61	0.47	0.33
2	1.33	1.11	1.08	0.60	1.04	.068	0.81	0.53	0.86	0.56	0.88	0.60
3	1.89	1.25	1.68	0.85	1.63	1.00	1.29	0.85	1.19	0.49	1.41	0.99
4	2.20	1.24	2.07	1.26	2.20	1.42	1.91	1.18	1.66	0.70	1.96	1.16
5	2.67	1.36	2.80	1.57	2.68	1.80	2.52	1.50	2.33	1.08	2.79	1.89
6	3.30	1.52	3.43	2.05	3.18	1.88	3.20	1.70	3.01	1.39	3.40	2.23
7	3.71	1.81	3.77	2.13	3.51	1.94	3.69	1.93	3.73	1.77	4.05	2.50
8	4.27	2.12	4.23	2.31	3.93	2.00	4.26	1.95	4.34	2.12	4.79	2.78
9	4.76	2.17	4.58	2.39	4.45	2.26	4.96	2.15	5.11	2.45	5.38	2.89
10	5.19	2.26	4.95	2.32	4.82	2.45	5.58	2.46	5.78	2.77	6.05	3.32
11	4.48	1.94	4.57	1.89	4.39	1.90	5.06	2.17	5.47	2.56	5.52	2.73
12	4.58	1.80	4.56	1.71	4.40	1.71	5.17	2.25	5.75	2.62	5.64	2.62
13	4.56	1.63	4.52	1.65	4.32	1.66	5.30	2.36	5.89	2.60	5.78	2.45
14	4.62	1.61	4.38	1.49	4.27	1.57	5.40	2.32	6.02	2.59	5.78	2.26
15	4.49	1.47	4.38	1.52	4.18	1.49	5.46	2.28	6.02	2.51	5.70	2.09
16	4.55	1.51	4.27	1.44	4.06	1.50	5.51	2.29	5.95	2.49	5.63	2.05
17	4.43	1.45	4.12	1.37	4.03	1.41	5.45	2.14	5.92	2.38	5.58	1.88
18	4.36	1.36	4.03	1.34	3.86	1.35	5.43	2.12	5.91	2.32	5.46	1.76
19	4.24	1.23	3.96	1.28	3.79	1.32	5.39	2.03	5.78	2.17	5.37	1.68
20	4.15	1.22	3.88	1.30	3.70	1.35	5.27	1.94	5.68	2.03	5.24	1.57

Table A4-3: Means and standard deviations (SD) for 1 MHz and 3 MHz ultrasound (1.0Watt/cm^2, 2X ERA) at slow (60 beats/min), moderate (120 beats/min) and fast (180 beats/min) movement speed of transducer at 2 cm below the skin's surface.

Time	1 MHz						3 MHz					
	Slow		Moderate		Fast		Slow		Moderate		Fast	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.33	0.26	0.41	0.44	0.16	0.20	0.18	0.30	0.44	0.71	0.24	0.15
2	0.63	0.37	0.58	0.49	0.46	0.30	0.27	0.38	0.58	0.67	0.35	0.22
3	0.81	0.43	0.79	0.43	0.64	0.39	0.53	0.74	0.67	0.56	0.51	0.33
4	1.00	0.56	0.99	0.60	0.84	0.53	0.68	0.76	0.83	0.67	0.73	0.59
5	1.24	0.65	1.30	0.54	1.11	0.67	0.84	1.05	1.13	0.81	1.00	0.93
6	1.44	0.77	1.62	0.73	1.40	0.78	1.13	1.22	1.39	0.94	1.23	1.33
7	1.78	0.94	1.93	0.86	1.61	0.82	1.54	1.54	1.62	1.18	1.62	1.69
8	2.04	1.11	2.13	0.97	1.94	0.84	1.71	1.75	2.01	1.52	2.04	2.10
9	2.44	1.14	2.47	1.08	2.14	1.00	2.15	2.09	2.38	1.80	2.32	2.42
10	2.74	1.27	2.77	1.07	2.32	1.03	2.57	2.26	2.70	2.02	2.75	2.76
11	2.54	1.22	2.64	1.03	2.31	0.95	2.45	2.28	2.77	2.04	2.61	2.40
12	2.71	1.25	2.69	1.05	2.40	0.97	2.58	2.43	2.96	2.13	2.79	2.49
13	2.84	1.22	2.86	1.05	2.48	1.03	2.88	2.41	3.20	2.14	3.12	2.36
14	2.87	1.24	2.89	1.04	2.53	0.92	3.02	2.50	3.28	2.17	3.16	2.46
15	3.01	1.23	2.97	1.07	2.53	0.93	3.14	2.52	3.42	2.16	3.30	2.50
16	3.08	1.25	2.99	1.06	2.60	1.01	3.27	2.56	3.53	2.20	3.28	2.45
17	3.12	1.21	2.95	1.09	2.60	1.06	3.37	2.48	3.66	2.11	3.46	2.41
18	3.14	1.17	3.01	1.05	2.59	1.00	3.43	2.50	3.79	2.02	3.52	2.32
19	3.11	1.15	3.04	1.00	2.65	1.00	3.52	2.44	3.77	2.01	3.52	2.26
20	3.16	1.12	3.03	1.05	2.63	1.01	3.52	2.44	3.81	1.98	3.56	2.18

Table A4-4: Means and standard deviations (SD) for 1 MHz and 3 MHz ultrasound (1.0Watt/cm^2, 2X ERA) at slow (60 beats/min), moderate (120 beats/min) and fast (180 beats/min) movement speed of transducer at 3 cm below the skin's surface.

Time	1 MHz						3 MHz					
	Slow		Moderate		Fast		Slow		Moderate		Fast	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.29	0.22	0.33	0.23	0.17	0.18	0.14	0.26	0.47	0.73	0.18	0.31
2	0.40	0.25	0.49	0.28	0.31	0.24	0.23	0.31	0.50	0.73	0.24	0.26
3	0.45	0.35	0.63	0.30	0.40	0.24	0.37	0.44	0.52	0.59	0.30	0.33
4	0.65	0.39	0.76	0.34	0.55	0.31	0.47	0.39	0.58	0.60	0.39	0.32
5	0.74	0.54	0.96	0.51	0.61	0.25	0.45	0.47	0.64	0.72	0.63	0.56
6	0.94	0.56	1.07	0.52	0.83	0.41	0.60	0.54	0.87	0.73	0.76	0.74
7	1.07	0.71	1.20	0.68	1.01	0.40	0.70	0.81	1.03	0.86	0.99	0.93
8	1.29	0.75	1.40	0.74	1.17	0.48	0.92	0.94	1.24	1.00	1.22	1.26
9	1.52	0.80	1.63	0.79	1.41	0.50	1.28	1.31	1.58	1.20	1.42	1.58
10	1.80	0.91	1.79	0.82	1.53	0.68	1.55	1.52	1.74	1.43	1.77	1.76
11	1.66	0.78	1.89	0.83	1.60	0.64	1.50	1.33	1.65	1.27	1.52	1.37
12	1.80	0.80	2.00	0.83	1.69	0.67	1.43	1.23	1.90	1.36	1.68	1.45
13	1.94	0.82	2.15	0.87	1.79	0.71	1.54	1.26	2.04	1.34	1.84	1.35
14	2.07	0.83	2.24	0.79	1.86	0.80	1.75	1.28	2.10	1.35	1.94	1.34
15	2.12	0.87	2.30	0.88	1.98	0.75	1.89	1.24	2.27	1.40	1.97	1.36
16	2.22	0.87	2.37	0.88	1.92	0.72	2.01	1.26	2.30	1.39	2.09	1.41
17	2.26	0.93	2.37	0.96	1.99	0.76	2.07	1.28	2.39	1.34	2.18	1.36
18	2.34	0.86	2.38	0.90	2.12	0.77	2.16	1.24	2.53	1.43	2.30	1.31
19	2.37	0.84	2.47	0.86	2.14	0.82	2.24	1.26	2.48	1.37	2.36	1.26
20	2.39	0.78	2.45	0.83	2.14	0.77	2.29	1.24	2.69	1.29	2.34	1.25

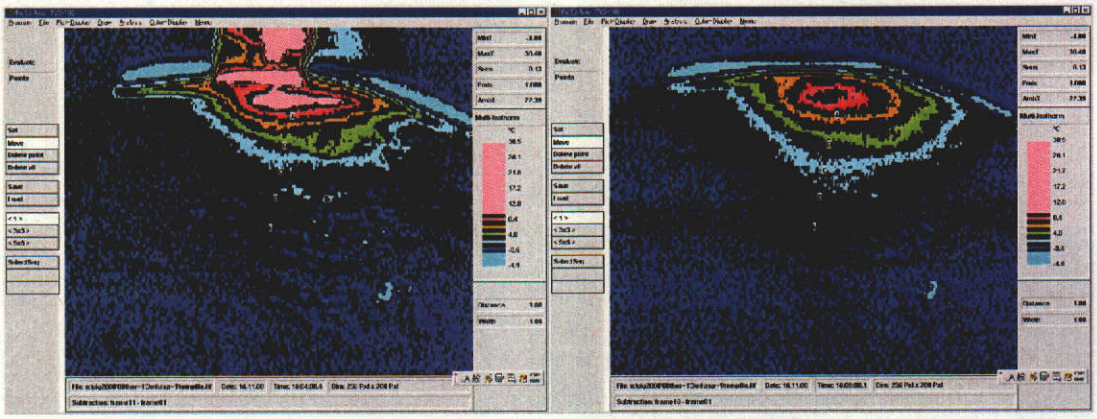
Table A4-5: Means and standard deviations (SD) for 1 MHz and 3 MHz ultrasound (1.0Watt/cm^2, 2X ERA) at slow (60 beats/min), moderate (120 beats/min) and fast (180 beats/min) movement speed of transducer at 4 cm below the skin's surface.

Time	1 MHz						3 MHz					
	Slow		Moderate		Fast		Slow		Moderate		Fast	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.19	0.22	0.24	0.20	0.22	0.22	0.12	0.24	0.26	0.68	0.05	0.16
2	0.33	0.19	0.31	0.26	0.37	0.21	0.07	0.14	0.36	0.67	0.17	0.21
3	0.39	0.17	0.49	0.25	0.41	0.26	0.19	0.20	0.35	0.54	0.21	0.28
4	0.50	0.29	0.50	0.24	0.45	0.31	0.18	0.17	0.44	0.56	0.27	0.30
5	0.55	0.27	0.69	0.34	0.52	0.25	0.18	0.26	0.49	0.63	0.33	0.36
6	0.58	0.32	0.74	0.37	0.67	0.39	0.33	0.22	0.51	0.61	0.41	0.38
7	0.77	0.34	0.88	0.31	0.81	0.34	0.33	0.27	0.59	0.72	0.57	0.44
8	0.91	0.40	1.02	0.45	0.95	0.35	0.40	0.27	0.63	0.64	0.55	0.48
9	1.08	0.46	1.16	0.47	1.09	0.41	0.54	0.32	0.74	0.64	0.74	0.58
10	1.27	0.52	1.28	0.43	1.21	0.46	0.54	0.41	0.87	0.70	0.85	0.54
11	1.32	0.62	1.38	0.56	1.28	0.55	0.50	0.44	0.90	0.64	0.80	0.49
12	1.31	0.60	1.40	0.51	1.34	0.52	0.63	0.47	1.02	0.61	0.97	0.66
13	1.49	0.65	1.52	0.45	1.40	0.51	0.69	0.47	1.06	0.67	0.99	0.68
14	1.58	0.67	1.55	0.48	1.53	0.46	0.83	0.51	1.18	0.62	1.07	0.56
15	1.64	0.64	1.62	0.51	1.52	0.46	0.86	0.53	1.18	0.67	1.11	0.66
16	1.65	0.59	1.68	0.55	1.60	0.53	0.92	0.50	1.20	0.68	1.22	0.64
17	1.69	0.59	1.76	0.54	1.61	0.48	1.02	0.37	1.33	0.69	1.26	0.70
18	1.83	0.68	1.74	0.56	1.63	0.47	1.10	0.46	1.45	0.63	1.36	0.69
19	1.87	0.70	1.83	0.55	1.66	0.50	1.14	0.47	1.47	0.69	1.34	0.64
20	1.89	0.67	1.81	0.55	1.78	0.52	1.19	0.43	1.55	0.66	1.40	0.60

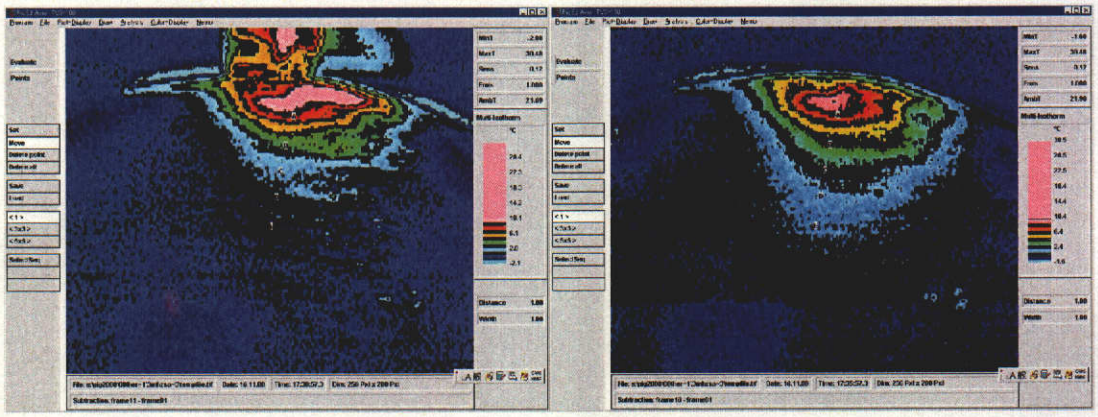
Table A4-6: Means and standard deviations (SD) for 1 MHz and 3 MHz ultrasound (1.0Watt/cm^2, 2X ERA) at slow (60 beats/min), moderate (120 beats/min) and fast (180 beats/min) movement speed of transducer at 5 cm below the skin's surface.

Time	1 MHz						3 MHz					
	Slow		Moderate		Fast		Slow		Moderate		Fast	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.16	0.21	0.26	0.17	0.14	0.15	0.00	0.19	0.40	0.72	0.21	0.18
2	0.23	0.20	0.33	0.30	0.24	0.08	0.02	0.16	0.47	0.59	0.30	0.20
3	0.33	0.25	0.37	0.31	0.35	0.18	0.10	0.29	0.39	0.56	0.34	0.30
4	0.36	0.34	0.42	0.26	0.42	0.15	0.15	0.28	0.42	0.55	0.41	0.32
5	0.47	0.40	0.63	0.35	0.43	0.14	0.15	0.33	0.54	0.66	0.44	0.28
6	0.56	0.36	0.62	0.17	0.56	0.33	0.17	0.30	0.59	0.58	0.53	0.36
7	0.62	0.40	0.77	0.30	0.62	0.33	0.19	0.33	0.65	0.59	0.60	0.42
8	0.76	0.42	0.84	0.39	0.74	0.30	0.24	0.40	0.69	0.61	0.63	0.41
9	0.89	0.45	0.97	0.38	0.92	0.35	0.37	0.41	0.74	0.59	0.76	0.56
10	1.04	0.50	1.09	0.40	0.99	0.50	0.45	0.43	0.80	0.67	0.80	0.54
11	1.06	0.55	1.16	0.37	0.94	0.50	0.41	0.38	0.84	0.67	0.78	0.52
12	1.16	0.47	1.23	0.39	1.04	0.45	0.31	0.40	0.87	0.64	0.82	0.55
13	1.17	0.56	1.25	0.36	1.15	0.49	0.39	0.40	0.92	0.63	0.93	0.56
14	1.19	0.58	1.27	0.33	1.16	0.36	0.49	0.41	0.95	0.64	0.89	0.58
15	1.33	0.54	1.30	0.39	1.22	0.41	0.51	0.45	0.97	0.68	0.89	0.51
16	1.37	0.57	1.34	0.28	1.20	0.38	0.47	0.48	0.97	0.70	0.99	0.57
17	1.39	0.48	1.27	0.42	1.26	0.40	0.58	0.42	1.02	0.67	1.00	0.54
18	1.42	0.48	1.42	0.39	1.20	0.36	0.62	0.48	1.11	0.71	1.07	0.58
19	1.44	0.50	1.38	0.37	1.30	0.35	0.63	0.47	1.10	0.69	1.02	0.57
20	1.55	0.53	1.42	0.38	1.36	0.65	0.64	0.41	1.14	0.60	1.10	0.56

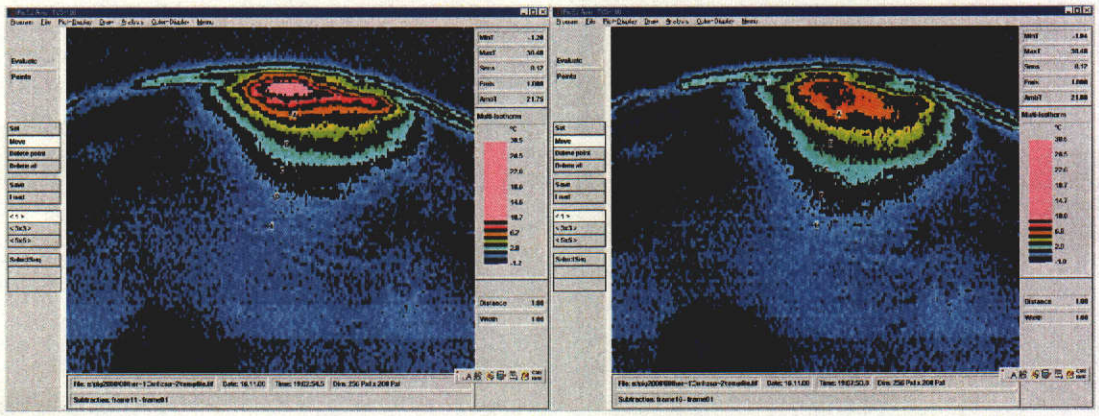
After 10 minutes **After 15 minutes**



slow movement speed of transducer (60 beats/min)



moderate movement speed of transducer (120 beats/min)



fast movement speed of transducer (180 beats/min)

Figure A4-1: Thermographic isothermic scans of specimens after 10 minutes exposure and 5 minutes post-exposure to 3MHz, 1.0 Watt/cm^2 (2X ERA) ultrasound at slow (60 beats/min), moderate (120 beats/min) and fast (180 beats/min) movement speed of transducer

TABLES OF MEANS AND THERMOGRAPHIC SCANS FOR CHAPER SIX

Table A5-1: Means and Standard Deviations (SD) for 1 MHz ultrasound (1.0Watt/cm2, moderate speed) for small (2X ERA), medium (3X ERA) and large (4X ERA) treatment area at skin's surface and 1 cm below skin surface

	Surface						1 cm					
	Small		Medium		Large		Small		Medium		Large	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	5.75	0.84	5.42	1.50	4.63	0.82	0.96	0.45	0.70	0.60	0.56	0.42
2	7.21	1.15	6.89	1.54	5.79	0.96	1.78	0.84	1.00	0.70	0.87	0.55
3	8.56	1.19	7.94	1.84	6.85	0.94	2.57	1.18	1.47	1.04	1.07	0.69
4	9.71	1.56	8.88	1.88	7.57	1.20	3.26	1.26	1.86	1.14	1.33	0.68
5	10.59	1.37	9.63	1.80	8.19	1.29	3.89	1.46	2.23	1.22	1.65	0.97
6	11.31	1.33	10.00	1.68	8.67	1.25	4.30	1.55	2.64	1.22	1.90	0.92
7	12.22	1.68	10.65	1.71	9.23	1.27	4.79	1.65	2.92	1.26	2.17	1.00
8	12.68	1.71	11.19	1.83	9.60	1.54	5.21	1.72	3.29	1.30	2.31	0.96
9	13.28	1.94	11.51	1.83	10.06	1.60	5.55	1.60	3.52	1.21	2.56	0.97
10	13.75	2.09	11.95	1.70	10.35	1.37	5.88	1.60	3.77	1.23	2.75	1.00
11	10.89	1.67	9.26	1.61	7.93	1.20	5.18	1.58	3.58	1.20	2.69	1.11
12	9.49	1.61	7.95	1.34	6.82	1.19	5.11	1.40	3.54	1.12	2.67	1.03
13	8.58	1.67	7.27	1.26	6.11	1.24	4.92	1.35	3.56	1.06	2.66	1.02
14	7.64	1.57	6.63	1.36	5.50	1.29	4.73	1.25	3.47	1.02	2.64	0.95
15	7.04	1.56	6.01	1.31	5.11	1.27	4.63	1.19	3.44	0.97	2.59	0.89
16	6.60	1.59	5.71	1.17	4.61	1.33	4.46	1.16	3.37	1.02	2.51	0.89
17	6.09	1.57	5.21	1.24	4.23	1.30	4.29	1.15	3.33	0.95	2.44	0.89
18	5.69	1.25	4.80	1.12	3.98	1.18	4.19	1.16	3.23	0.93	2.40	0.84
19	5.29	1.34	4.34	1.10	3.71	1.15	3.97	1.08	3.14	0.91	2.35	0.81
20	5.06	1.39	4.13	1.05	3.38	1.07	3.81	1.13	3.10	0.92	2.20	0.86

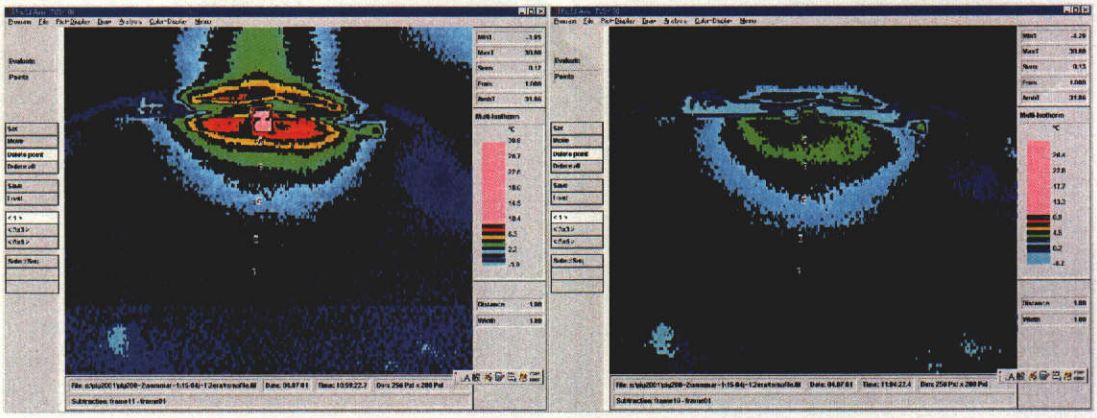
Table A5-2: Means and Standard Deviations (SD) for 1 MHz ultrasound (1.0Watt/cm2, moderate speed) for small (2X ERA), medium (3X ERA) and large (4X ERA) treatment area at 2 cm and 3 cm below skin surface

	2 cm						3 cm					
	Small		Medium		Large		Small		Medium		Large	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.51	0.39	0.36	0.30	0.29	0.29	0.25	0.43	0.22	0.23	0.08	0.15
2	0.74	0.58	0.50	0.45	0.43	0.47	0.30	0.44	0.28	0.26	0.20	0.16
3	1.04	0.74	0.71	0.55	0.54	0.50	0.43	0.44	0.43	0.28	0.23	0.26
4	1.37	0.75	0.85	0.66	0.65	0.69	0.66	0.51	0.44	0.41	0.34	0.28
5	1.74	1.00	1.09	0.79	0.91	0.94	0.82	0.71	0.59	0.44	0.44	0.39
6	2.07	1.17	1.23	0.85	0.96	0.88	1.05	0.77	0.66	0.49	0.45	0.40
7	2.37	1.29	1.53	0.99	1.14	1.00	1.23	0.88	0.82	0.47	0.63	0.54
8	2.60	1.21	1.75	1.11	1.21	1.03	1.44	0.90	0.98	0.54	0.70	0.61
9	2.94	1.36	1.96	1.20	1.39	1.04	1.64	1.03	1.10	0.60	0.82	0.64
10	3.25	1.48	2.19	1.21	1.65	1.07	1.88	1.12	1.22	0.68	0.96	0.69
11	2.90	1.20	2.01	1.09	1.60	1.16	1.80	1.08	1.27	0.64	0.96	0.64
12	2.99	1.09	2.12	1.12	1.59	1.15	1.89	1.05	1.29	0.70	0.97	0.65
13	3.02	1.03	2.21	1.15	1.62	1.14	1.98	1.13	1.43	0.68	1.01	0.71
14	3.09	1.03	2.24	1.15	1.64	1.06	2.00	1.02	1.45	0.71	1.05	0.67
15	3.07	0.98	2.25	1.11	1.71	0.97	2.06	0.99	1.51	0.70	1.04	0.65
16	3.07	0.93	2.30	1.15	1.73	0.96	2.10	0.91	1.55	0.70	1.11	0.65
17	3.05	1.00	2.38	1.21	1.74	0.92	2.07	0.96	1.58	0.74	1.16	0.70
18	3.11	1.02	2.39	1.13	1.71	0.87	2.15	0.92	1.63	0.70	1.21	0.71
19	3.01	0.91	2.33	1.11	1.76	0.84	2.11	0.89	1.63	0.77	1.25	0.74
20	2.95	0.95	2.36	1.14	1.71	0.81	2.15	0.84	1.70	0.74	1.17	0.68

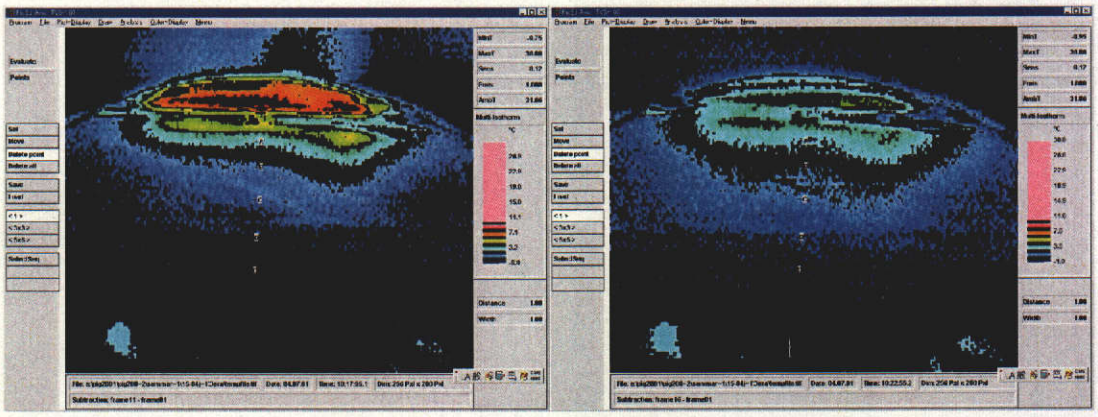
Table A5-3: Means and Standard Deviations (SD) for 1 MHz ultrasound (1.0Watt/cm2, moderate speed) for small (2X ERA), medium (3X ERA) and large (4X ERA) treatment area at 4 cm and 5 cm below skin surface

	4 cm						5 cm					
	Small		Medium		Large		Small		Medium		Large	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.16	0.30	0.11	0.24	0.06	0.19	0.06	0.26	0.12	0.21	0.04	0.16
2	0.24	0.32	0.12	0.28	0.16	0.24	0.09	0.35	0.06	0.32	0.07	0.21
3	0.33	0.40	0.21	0.31	0.17	0.30	0.19	0.40	0.16	0.33	0.09	0.27
4	0.41	0.46	0.26	0.39	0.23	0.36	0.27	0.39	0.16	0.42	0.09	0.29
5	0.55	0.61	0.34	0.43	0.29	0.46	0.35	0.61	0.30	0.41	0.18	0.36
6	0.70	0.72	0.47	0.44	0.35	0.45	0.52	0.64	0.32	0.39	0.20	0.39
7	0.88	0.72	0.57	0.47	0.40	0.48	0.65	0.74	0.43	0.45	0.30	0.40
8	0.97	0.80	0.62	0.51	0.42	0.56	0.67	0.78	0.50	0.54	0.31	0.49
9	1.13	0.91	0.73	0.54	0.51	0.57	0.81	0.85	0.56	0.60	0.40	0.54
10	1.26	1.02	0.81	0.66	0.59	0.61	0.91	0.97	0.65	0.63	0.41	0.58
11	1.29	0.98	0.89	0.65	0.66	0.59	0.93	0.99	0.69	0.68	0.45	0.57
12	1.37	0.97	0.90	0.69	0.60	0.58	0.99	1.02	0.65	0.65	0.45	0.64
13	1.35	0.99	0.95	0.72	0.64	0.65	1.01	0.99	0.76	0.69	0.45	0.61
14	1.41	0.93	1.01	0.69	0.69	0.65	1.03	0.92	0.75	0.72	0.46	0.62
15	1.41	0.91	1.05	0.68	0.70	0.66	1.07	0.86	0.79	0.67	0.50	0.64
16	1.51	0.92	1.10	0.66	0.74	0.66	1.12	0.93	0.84	0.67	0.53	0.61
17	1.50	0.93	1.14	0.77	0.84	0.72	1.06	0.90	0.84	0.73	0.60	0.65
18	1.53	0.95	1.14	0.70	0.83	0.71	1.17	0.82	0.84	0.66	0.58	0.68
19	1.59	0.87	1.15	0.78	0.85	0.73	1.08	0.83	0.85	0.76	0.60	0.68
20	1.53	0.86	1.22	0.77	0.86	0.73	1.08	0.79	0.91	0.71	0.65	0.68

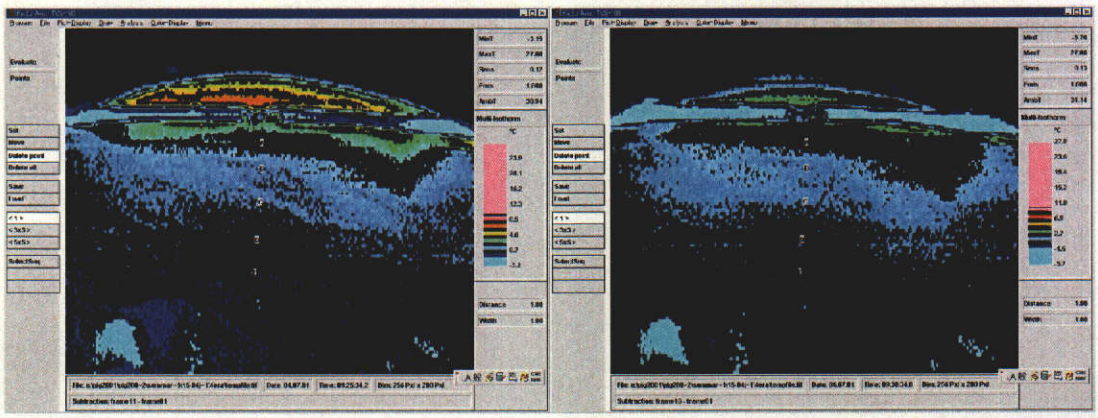
10 minutes **15 minutes**



Small (2X ERA)



Medium (3X ERA)



Large (4X ERA)

Figure A5-1: Thermographic isothermic scans of specimens after 10 minutes exposure and 5 minutes post-exposure to 1MHz, 1.0 Watt/cm^2 (moderate speed) ultrasound for small (2X ERA), medium (3X ERA) and large (4X ERA) treatment areas.

Appendix 6

PATHOLOGY REPORT FOR POST-MORTEM PIG TISSUES EXPOSED TO THERAPEUTIC ULTRASOUND

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(Co-ordinator, ADT Project (Bibliographic Services), Curtin University of Technology, 12/12/03)

Appendix 7

PATHOLOGY REPORT FOR POST-MORTEM PIG TISSUES EXPOSED TO MICROWAVE DIATHERMY

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(Co-ordinator, ADT Project (Bibliographic Services), Curtin University of Technology, 12/12/03)

TABLES OF MEANS AND THERMOGRAPHIC SCANS FOR CHAPTER EIGHT

Table A8-1: Means and Standard Deviations (SD) for 1 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.1Watt/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	2.30	0.71	0.27	0.21	0.26	0.20	0.15	0.12	0.04	0.16	0.00	0.12
2	2.32	0.72	0.33	0.23	0.18	0.17	0.17	0.20	0.05	0.18	0.11	0.16
3	2.48	0.86	0.54	0.26	0.25	0.08	0.20	0.13	0.11	0.20	0.07	0.15
4	2.70	0.91	0.69	0.32	0.37	0.18	0.31	0.15	0.09	0.21	0.14	0.12
5	2.80	1.00	0.93	0.27	0.45	0.13	0.29	0.05	0.14	0.20	0.08	0.16
6	2.96	1.17	0.97	0.41	0.38	0.23	0.24	0.31	0.16	0.25	0.10	0.16
7	3.00	1.23	1.20	0.40	0.54	0.27	0.37	0.21	0.16	0.30	0.24	0.16
8	2.94	1.22	1.25	0.54	0.61	0.40	0.42	0.29	0.28	0.32	0.27	0.15
9	3.22	1.18	1.38	0.46	0.68	0.20	0.47	0.31	0.33	0.27	0.25	0.19
10	3.16	1.31	1.38	0.50	0.70	0.27	0.45	0.33	0.22	0.33	0.16	0.23
11	2.68	1.27	1.26	0.46	0.52	0.28	0.40	0.29	0.22	0.25	0.17	0.24
12	2.54	1.50	1.31	0.63	0.57	0.38	0.44	0.36	0.21	0.41	0.21	0.23
13	2.38	1.38	1.26	0.62	0.73	0.32	0.51	0.31	0.36	0.28	0.33	0.22
14	2.30	1.28	1.21	0.67	0.73	0.32	0.51	0.32	0.31	0.38	0.28	0.28
15	2.18	1.47	1.09	0.80	0.63	0.34	0.49	0.37	0.30	0.34	0.23	0.27
16	1.94	1.44	1.11	0.61	0.70	0.26	0.50	0.44	0.32	0.39	0.21	0.17
17	1.78	1.54	1.03	0.66	0.69	0.26	0.47	0.30	0.31	0.32	0.30	0.20
18	1.44	1.35	0.91	0.64	0.66	0.31	0.44	0.39	0.27	0.32	0.27	0.17
19	1.44	1.48	0.98	0.73	0.56	0.36	0.48	0.38	0.21	0.28	0.17	0.19
20	1.12	1.37	0.92	0.65	0.68	0.34	0.57	0.25	0.30	0.33	0.24	0.19

Table A8-2: Means and Standard Deviations (SD) for 1 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.3Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	3.38	1.27	0.41	0.30	0.16	0.21	0.00	0.15	-0.17	0.18	-0.05	0.06
2	4.14	1.12	0.66	0.34	0.17	0.27	0.14	0.15	0.07	0.08	0.04	0.17
3	4.56	1.17	0.83	0.31	0.25	0.23	0.09	0.07	0.10	0.12	0.01	0.19
4	5.04	1.15	1.06	0.35	0.38	0.35	0.26	0.12	0.19	0.13	0.04	0.08
5	5.60	1.18	1.30	0.43	0.46	0.27	0.23	0.20	0.20	0.13	0.12	0.16
6	5.98	1.23	1.63	0.37	0.72	0.43	0.37	0.13	0.31	0.18	0.18	0.22
7	6.42	1.23	1.89	0.36	0.88	0.48	0.54	0.09	0.33	0.29	0.28	0.23
8	6.76	1.27	2.15	0.34	1.08	0.41	0.54	0.16	0.38	0.26	0.27	0.24
9	7.00	1.36	2.29	0.46	1.16	0.40	0.63	0.19	0.56	0.35	0.26	0.39
10	7.30	1.29	2.45	0.47	1.33	0.53	0.68	0.23	0.52	0.32	0.36	0.23
11	6.24	1.21	2.21	0.29	1.13	0.53	0.71	0.14	0.48	0.28	0.36	0.23
12	5.50	1.06	2.30	0.33	1.20	0.62	0.76	0.17	0.51	0.30	0.34	0.25
13	4.84	0.98	2.41	0.41	1.42	0.57	0.81	0.29	0.53	0.31	0.36	0.39
14	4.48	0.91	2.33	0.38	1.34	0.60	0.76	0.35	0.52	0.34	0.31	0.35
15	4.28	0.93	2.35	0.37	1.53	0.60	0.86	0.23	0.62	0.37	0.38	0.33
16	4.02	0.82	2.25	0.39	1.42	0.52	0.84	0.26	0.62	0.32	0.38	0.40
17	3.68	0.82	2.29	0.48	1.42	0.58	0.85	0.29	0.64	0.52	0.37	0.37
18	3.58	0.98	2.13	0.45	1.46	0.45	0.89	0.29	0.60	0.39	0.38	0.35
19	3.40	0.82	2.10	0.40	1.38	0.49	0.95	0.29	0.68	0.32	0.49	0.28
20	3.24	0.93	1.99	0.47	1.35	0.58	0.93	0.31	0.64	0.40	0.42	0.30

Table A8-3: Means and Standard Deviations (SD) for 1 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.5Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	3.58	0.87	0.38	0.31	0.41	0.30	-0.02	0.13	0.10	0.06	0.13	0.12
2	4.78	0.86	0.78	0.33	0.52	0.35	0.04	0.11	0.14	0.19	0.15	0.09
3	5.98	0.89	1.19	0.39	0.67	0.56	0.26	0.10	0.23	0.11	0.15	0.08
4	6.68	0.82	1.51	0.35	0.99	0.44	0.39	0.30	0.40	0.10	0.21	0.18
5	7.52	0.80	1.93	0.45	1.22	0.61	0.47	0.29	0.46	0.22	0.37	0.11
6	8.22	0.99	2.42	0.53	1.44	0.70	0.69	0.34	0.56	0.14	0.41	0.10
7	8.86	0.83	2.71	0.53	1.67	0.73	0.87	0.29	0.56	0.25	0.41	0.21
8	9.36	0.96	3.23	0.69	2.04	0.89	0.96	0.43	0.64	0.25	0.59	0.12
9	9.88	1.19	3.66	0.78	2.30	0.97	1.16	0.35	0.78	0.24	0.65	0.32
10	10.30	1.09	4.05	0.80	2.53	1.10	1.35	0.48	0.99	0.36	0.72	0.14
11	8.80	0.86	3.68	0.82	2.44	0.84	1.27	0.44	0.88	0.31	0.68	0.16
12	7.50	0.71	3.80	0.81	2.48	1.05	1.45	0.40	1.04	0.28	0.83	0.20
13	7.06	0.65	3.76	0.75	2.66	0.92	1.49	0.47	1.03	0.37	0.80	0.25
14	6.52	0.70	3.66	0.61	2.64	0.91	1.63	0.57	1.07	0.50	0.85	0.25
15	5.92	0.61	3.59	0.72	2.61	0.96	1.62	0.52	1.13	0.43	0.86	0.38
16	5.46	0.89	3.46	0.62	2.74	0.95	1.60	0.43	1.11	0.45	0.80	0.43
17	4.92	0.82	3.41	0.69	2.62	0.80	1.56	0.41	1.03	0.34	0.79	0.30
18	4.74	0.71	3.31	0.80	2.54	0.72	1.61	0.34	1.23	0.35	0.84	0.29
19	4.38	0.75	3.28	0.80	2.49	0.79	1.59	0.47	1.21	0.39	0.89	0.24
20	4.10	0.83	3.03	0.74	2.53	0.83	1.76	0.55	1.17	0.44	0.91	0.36

Table A8-4: Means and Standard Deviations (SD) for 1 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.7Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	4.36	0.46	0.16	0.25	0.21	0.05	0.13	0.10	0.09	0.19	0.04	0.19
2	5.76	0.77	0.46	0.35	0.36	0.18	0.17	0.14	0.14	0.17	0.13	0.17
3	6.96	1.34	0.99	0.35	0.51	0.33	0.39	0.16	0.21	0.20	0.14	0.15
4	7.84	1.42	1.47	0.40	0.81	0.28	0.44	0.13	0.26	0.14	0.36	0.36
5	8.48	1.56	2.21	0.58	1.09	0.31	0.53	0.28	0.38	0.23	0.26	0.20
6	9.20	1.70	2.45	0.65	1.33	0.43	0.66	0.30	0.50	0.23	0.44	0.18
7	9.96	1.83	2.90	0.80	1.58	0.54	0.94	0.35	0.59	0.23	0.51	0.28
8	10.60	1.96	3.18	0.92	1.88	0.55	0.98	0.36	0.79	0.35	0.64	0.37
9	11.22	2.23	3.57	1.09	2.24	0.60	1.26	0.33	0.82	0.30	0.71	0.36
10	11.66	2.51	4.01	1.11	2.52	0.80	1.44	0.44	1.03	0.41	0.79	0.30
11	10.02	2.15	3.75	1.28	2.25	0.70	1.45	0.32	1.06	0.45	0.83	0.29
12	9.08	2.04	3.88	1.25	2.36	0.54	1.41	0.47	0.99	0.34	0.83	0.34
13	8.48	1.77	3.97	1.23	2.56	0.58	1.54	0.46	1.12	0.43	0.84	0.46
14	8.10	1.69	4.03	1.28	2.56	0.72	1.75	0.52	1.29	0.47	0.98	0.38
15	7.56	1.48	3.99	1.29	2.55	0.68	1.77	0.40	1.23	0.36	0.89	0.34
16	7.16	1.55	3.94	1.18	2.53	0.66	1.79	0.33	1.31	0.37	0.97	0.41
17	7.06	1.71	3.76	1.33	2.55	0.72	1.77	0.32	1.27	0.41	1.00	0.44
18	6.62	1.47	3.75	1.23	2.61	0.72	1.78	0.37	1.26	0.40	0.98	0.39
19	6.30	1.40	3.78	1.25	2.61	0.67	1.85	0.30	1.26	0.40	1.08	0.41
20	6.02	1.31	3.70	1.12	2.55	0.63	1.79	0.47	1.30	0.40	1.11	0.54

Table A8-5: Means and Standard Deviations (SD) for 1 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.9Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	5.46	1.41	0.50	0.36	0.40	0.23	0.11	0.11	0.19	0.07	0.03	0.20
2	7.44	1.35	0.92	0.45	0.73	0.40	0.27	0.17	0.11	0.23	0.18	0.18
3	9.18	2.22	1.50	0.54	1.01	0.57	0.42	0.17	0.39	0.25	0.20	0.29
4	10.20	2.22	2.07	0.68	1.23	0.41	0.62	0.23	0.48	0.33	0.36	0.25
5	11.12	2.68	2.68	0.89	1.60	0.54	0.83	0.24	0.63	0.32	0.42	0.38
6	11.78	2.19	3.33	0.86	2.07	0.55	1.04	0.25	0.84	0.24	0.56	0.36
7	12.50	2.18	3.81	0.88	2.45	0.69	1.14	0.32	0.92	0.40	0.67	0.37
8	13.16	2.42	4.36	0.96	2.74	0.64	1.42	0.31	1.17	0.41	0.86	0.41
9	13.94	2.28	4.97	1.16	3.14	0.62	1.68	0.35	1.31	0.46	0.97	0.50
10	14.58	1.99	5.28	1.13	3.39	0.59	1.94	0.35	1.41	0.30	1.09	0.43
11	12.18	1.61	4.91	0.94	3.14	0.55	1.95	0.32	1.46	0.53	1.17	0.47
12	10.76	1.45	5.09	0.84	3.29	0.56	2.11	0.31	1.49	0.43	1.21	0.41
13	9.76	1.15	5.19	0.69	3.40	0.56	2.12	0.34	1.68	0.46	1.15	0.50
14	9.00	1.16	5.28	0.77	3.40	0.46	2.18	0.26	1.66	0.46	1.10	0.47
15	8.46	1.22	5.38	0.71	3.65	0.73	2.30	0.36	1.70	0.39	1.35	0.37
16	8.00	1.20	5.30	0.55	3.61	0.68	2.34	0.43	1.78	0.41	1.36	0.44
17	7.64	.95	5.29	0.63	3.46	0.64	2.37	0.30	1.70	0.51	1.34	0.48
18	7.28	1.03	5.14	0.63	3.53	0.59	2.34	0.29	1.77	0.49	1.30	0.41
19	6.82	1.18	5.05	0.64	3.57	0.57	2.35	0.41	1.85	0.55	1.30	0.48
20	6.54	1.11	4.89	0.54	3.54	0.58	2.46	0.28	1.87	0.45	1.38	0.45

Table A8-6: Means and Standard Deviations (SD) for 1 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 1.1 Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	5.98	1.52	0.54	0.34	0.68	0.45	0.09	0.32	0.12	0.27	0.42	0.59
2	8.06	1.54	1.01	0.20	0.98	0.73	0.24	0.20	0.24	0.25	0.10	0.28
3	9.50	1.68	1.48	0.30	1.33	0.83	0.46	0.27	0.28	0.24	0.23	0.28
4	10.78	1.89	1.98	0.35	1.64	1.01	0.53	0.28	0.41	0.38	0.41	0.27
5	11.76	1.83	2.68	0.66	2.05	1.15	0.86	0.37	0.49	0.41	0.43	0.44
6	12.66	1.99	3.32	0.74	2.56	1.13	1.10	0.63	0.73	0.51	0.69	0.51
7	13.98	2.46	4.00	0.91	2.85	1.36	1.38	0.46	0.89	0.62	0.76	0.65
8	14.66	2.43	4.54	10.08	3.36	1.42	1.59	0.61	1.08	0.60	1.05	0.65
9	15.34	2.51	5.04	1.28	3.76	1.40	1.86	0.78	1.37	0.65	1.17	0.79
10	15.88	2.70	5.49	1.39	4.03	1.76	2.13	0.85	1.48	0.87	1.33	0.94
11	12.88	2.14	5.20	1.41	3.84	1.52	2.23	0.86	1.51	0.90	1.30	0.92
12	11.30	2.17	5.27	1.29	3.86	1.40	2.39	0.84	1.75	0.93	1.48	0.94
13	10.40	1.76	5.36	1.39	4.12	1.18	2.44	0.85	1.79	0.99	1.47	0.93
14	9.70	1.97	5.33	1.35	4.18	1.13	2.46	0.91	1.85	0.88	1.60	0.80
15	8.92	1.81	5.33	1.35	4.20	1.31	2.63	0.85	1.89	0.95	1.56	0.88
16	8.16	1.72	5.20	1.34	4.23	1.28	2.67	0.85	1.86	0.98	1.60	0.91
17	7.60	1.67	5.06	1.42	4.15	1.37	2.64	0.91	2.00	0.96	1.61	0.99
18	7.06	1.50	5.04	1.23	4.17	1.18	2.81	0.86	2.02	0.94	1.71	01.03
19	6.52	1.40	4.81	1.33	4.22	1.14	2.78	0.71	1.99	0.95	1.66	0.89
20	6.12	1.28	4.59	1.31	4.19	1.22	2.68	0.94	2.10	0.96	1.71	0.98

Table A8-7: Means and Standard Deviations (SD) for 1 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 1.3Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	6.04	1.48	0.64	0.44	0.68	0.33	0.20	0.24	0.10	0.18	0.15	0.23
2	8.68	1.82	1.14	0.65	0.93	0.69	0.20	0.16	0.11	0.09	0.22	0.14
3	10.40	1.91	1.74	0.73	1.15	.60	0.36	0.33	0.19	0.10	0.33	0.18
4	11.84	2.16	2.64	0.79	1.75	0.70	0.74	0.44	0.43	0.24	0.38	0.10
5	12.88	2.32	3.29	1.02	1.98	0.82	0.96	0.54	0.49	0.26	0.52	0.09
6	13.84	2.27	4.07	1.42	2.53	1.06	1.19	0.54	0.71	0.25	0.67	0.25
7	14.86	2.84	4.61	1.27	2.97	1.05	1.49	0.74	1.00	0.34	0.84	0.41
8	15.62	2.74	5.20	1.73	3.38	1.06	1.73	0.68	1.17	0.32	1.02	0.28
9	16.34	2.91	5.69	1.95	3.82	1.42	2.08	0.67	1.30	0.44	1.08	0.40
10	16.38	3.02	6.09	1.93	4.34	1.31	2.39	0.87	1.51	0.40	1.31	0.38
11	13.90	2.77	5.91	1.81	3.78	1.18	2.30	0.92	1.62	0.52	1.34	0.45
12	12.44	2.60	5.84	1.83	3.87	1.02	2.46	0.79	1.68	0.46	1.45	0.41
13	11.36	2.57	5.93	1.85	3.95	1.11	2.62	0.72	1.72	0.48	1.58	0.43
14	10.62	2.57	6.02	1.96	4.05	0.94	2.72	0.77	1.97	0.44	1.64	0.42
15	9.70	2.43	6.09	1.70	4.22	1.01	2.88	0.70	2.00	0.46	1.74	0.50
16	8.96	2.23	5.97	1.92	4.19	1.01	2.83	0.78	2.10	0.46	1.77	0.43
17	8.40	2.22	5.89	2.04	4.28	0.90	2.98	0.71	2.11	0.51	1.79	0.46
18	7.92	2.19	5.63	2.02	4.13	0.84	2.89	0.64	2.05	0.46	1.90	0.40
19	7.40	2.08	5.54	2.08	4.23	0.98	2.89	0.69	2.21	0.47	1.78	0.40
20	6.96	2.04	5.41	2.05	4.21	0.96	2.93	0.67	2.20	0.51	1.81	0.47

Table A8-8: Means and Standard Deviations (SD) for 1 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 1.5 Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	7.78	1.59	0.62	0.25	0.37	0.30	0.22	0.16	0.28	0.22	0.21	0.16
2	10.64	2.16	1.24	0.18	0.79	0.71	0.39	0.34	0.41	0.25	0.23	0.27
3	12.74	2.33	2.29	0.50	1.16	0.46	0.60	0.49	0.48	0.50	0.45	0.24
4	14.74	2.22	2.92	0.48	1.64	0.79	0.95	0.54	0.69	0.61	0.55	0.44
5	16.12	2.37	3.96	0.75	2.22	0.90	1.30	0.72	1.13	0.72	0.83	0.49
6	17.46	2.41	4.61	0.76	2.60	1.09	1.78	0.85	1.32	0.87	1.07	0.51
7	18.56	2.51	5.33	0.73	3.27	1.09	2.09	1.02	1.72	0.99	1.22	0.78
8	19.54	2.33	6.29	0.88	3.81	1.18	2.56	1.08	1.96	01.09	1.52	0.98
9	20.16	2.21	6.77	0.89	4.28	1.48	3.01	1.35	2.23	1.37	1.80	0.96
10	20.86	2.05	7.19	0.88	4.70	1.54	3.36	1.42	2.64	1.34	2.05	1.09
11	16.90	0.86	6.53	0.78	4.71	1.47	3.38	1.38	2.65	1.46	2.18	1.03
12	14.92	0.65	6.78	0.78	4.95	1.29	3.62	1.26	2.89	1.33	2.21	1.12
13	13.28	0.73	6.72	0.75	5.00	1.28	3.73	1.29	2.92	1.37	2.24	1.05
14	12.34	0.50	6.86	0.80	5.30	1.26	3.85	1.22	2.92	1.31	2.29	1.14
15	11.36	0.44	6.87	0.82	5.24	1.24	3.90	1.27	3.01	1.39	2.39	1.06
16	10.60	0.40	6.79	0.90	5.28	1.17	3.92	1.11	3.03	1.32	2.39	1.04
17	9.90	0.31	6.66	0.92	5.26	1.28	3.82	1.12	3.03	1.25	2.31	1.08
18	9.32	0.43	6.46	0.97	5.23	1.15	3.87	1.02	3.09	1.09	2.37	1.02
19	8.74	0.49	6.36	0.91	5.26	1.02	3.97	0.99	3.11	1.02	2.40	1.03
20	8.36	0.59	6.17	1.03	5.23	0.93	3.96	0.92	3.06	1.09	2.36	1.02

Table A8-9: Means and Standard Deviations (SD) for 3 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.1 Watt/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	2.80	1.06	0.14	0.13	-0.03	0.13	-0.05	0.23	0.01	0.19	-0.01	2.80
2	3.16	.93	0.46	0.18	0.12	0.17	0.01	0.31	-0.01	0.16	0.01	3.16
3	3.42	1.00	0.56	0.28	0.14	0.15	0.11	0.25	0.01	0.05	0.01	3.42
4	3.66	1.32	0.66	0.31	0.23	0.19	0.11	0.17	0.05	0.10	0.05	3.66
5	3.82	1.19	1.00	0.29	0.36	0.14	0.24	0.26	0.13	0.17	0.02	3.82
6	4.02	1.32	1.06	0.29	0.30	0.23	0.06	0.19	0.07	0.18	0.04	4.02
7	4.10	1.22	1.40	0.46	0.46	0.30	0.19	0.33	0.12	0.22	0.12	4.10
8	4.22	1.30	1.47	0.25	0.64	0.27	0.30	0.37	0.17	0.13	0.20	4.22
9	4.32	1.35	1.50	0.35	0.68	0.28	0.35	0.36	0.17	0.20	0.14	4.32
10	4.54	1.38	1.63	0.34	0.64	0.29	0.38	0.38	0.20	0.11	0.12	4.54
11	4.04	1.40	1.48	0.25	0.63	0.24	0.30	0.40	0.18	0.21	0.13	4.04
12	3.38	1.33	1.41	0.19	0.68	0.30	0.31	0.30	0.12	0.07	0.13	3.38
13	3.14	1.28	1.44	0.21	0.71	0.29	0.38	0.45	0.20	0.18	0.13	3.14
14	2.86	1.22	1.54	0.23	0.69	0.27	0.48	0.39	0.28	0.19	0.26	2.86
15	2.70	1.32	1.42	0.15	0.72	0.18	0.41	0.41	0.23	0.20	0.14	2.70
16	2.42	1.32	1.33	0.23	0.65	0.28	0.40	0.27	0.20	0.12	0.24	2.42
17	2.16	1.33	1.38	0.24	0.74	0.30	0.38	0.50	0.24	0.22	0.17	2.16
18	1.92	1.30	1.36	0.31	0.84	0.19	0.56	0.48	0.28	0.16	0.25	1.92
19	1.74	1.20	1.14	0.23	0.70	0.22	0.46	0.42	0.26	0.24	0.16	1.74
20	1.54	1.33	1.09	0.35	0.59	0.24	0.42	0.50	0.19	0.19	0.20	1.54

Table A8-10: Means and Standard Deviations (SD) for 3 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.3 Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	4.74	1.54	0.30	0.26	0.07	0.08	0.13	0.20	-0.10	0.25	-0.04	0.23
2	5.90	1.89	0.73	0.42	0.23	0.14	0.28	0.30	-0.06	0.30	-0.02	0.18
3	6.68	1.79	1.12	0.72	0.19	0.22	0.23	0.22	0.10	0.10	-0.02	0.23
4	7.52	1.83	1.51	0.83	0.38	0.23	0.26	0.41	0.02	0.17	.000	0.22
5	8.22	2.20	1.85	0.87	0.50	0.14	0.32	0.37	0.08	0.21	-0.02	0.23
6	8.96	2.31	2.20	0.83	0.65	0.28	0.40	0.34	0.13	0.19	0.07	0.27
7	9.94	3.05	2.30	1.30	0.80	0.38	0.43	0.39	0.11	0.30	0.04	0.32
8	10.30	3.03	2.76	1.15	1.03	0.54	0.59	0.33	0.18	0.39	0.18	0.34
9	11.08	3.19	3.19	1.10	1.13	0.38	0.49	0.44	0.17	0.41	0.18	0.39
10	11.26	2.76	3.48	1.11	1.26	0.45	0.58	0.43	0.19	0.30	0.16	0.30
11	9.76	2.40	3.29	0.95	1.22	0.32	0.61	0.48	0.26	0.44	0.10	0.43
12	8.84	1.80	3.34	0.97	1.30	0.44	0.76	0.44	0.36	0.36	0.14	0.36
13	7.78	1.25	3.29	1.02	1.36	0.47	0.77	0.43	0.40	0.42	0.26	0.45
14	7.42	1.30	3.36	0.82	1.44	0.45	0.82	0.40	0.43	0.42	0.25	0.51
15	6.96	1.40	3.28	0.68	1.52	0.41	0.95	0.44	0.43	0.48	0.18	0.45
16	6.60	1.29	3.24	0.60	1.51	0.42	0.86	0.50	0.38	0.53	0.26	0.46
17	6.18	1.10	3.12	0.60	1.56	0.39	0.98	0.43	0.56	0.49	0.25	0.43
18	5.58	1.22	3.11	0.51	1.60	0.34	1.00	0.41	0.59	0.42	0.46	0.44
19	5.22	1.15	3.00	0.52	1.63	0.37	1.15	0.47	0.62	0.43	0.42	0.56
20	4.96	1.09	2.85	0.40	1.62	0.39	1.01	0.60	0.50	0.50	0.48	0.61

Table A8-11: Means and Standard Deviations (SD) for 3 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.5 Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	5.82	1.98	0.35	0.27	0.07	0.16	0.08	0.21	0.08	0.18	0.16	0.23
2	7.32	2.01	0.71	0.23	0.24	0.26	0.25	0.27	0.19	0.25	0.14	0.34
3	8.90	1.90	1.02	0.29	0.34	0.27	0.20	0.23	0.26	0.24	0.20	0.27
4	9.52	1.75	1.50	0.40	0.34	0.23	0.28	0.26	0.23	0.25	0.20	0.29
5	10.98	2.01	1.80	0.66	0.44	0.30	0.40	0.29	0.31	0.25	0.25	0.29
6	11.64	1.95	2.42	0.72	0.55	0.50	0.37	0.34	0.32	0.28	0.26	0.27
7	12.64	2.19	2.77	0.62	0.74	0.40	0.36	0.37	0.31	0.39	0.24	0.34
8	13.30	2.00	3.14	0.78	1.06	0.45	0.48	0.37	0.37	0.40	0.38	0.35
9	13.72	2.07	3.53	0.95	1.07	0.46	0.56	0.43	0.49	0.39	0.32	0.32
10	14.18	2.48	3.95	0.95	1.22	0.47	0.59	0.43	0.42	0.42	0.37	0.39
11	12.38	1.90	3.82	0.88	1.26	0.69	0.67	0.45	0.49	0.49	0.47	0.53
12	10.74	1.91	3.78	0.85	1.40	0.62	0.74	0.50	0.43	0.55	0.37	0.48
13	9.68	1.97	3.86	0.84	1.58	0.65	0.88	0.53	0.55	0.45	0.40	0.55
14	8.94	1.87	3.94	0.75	1.63	0.68	1.04	0.46	0.60	0.57	0.42	0.57
15	8.20	1.84	4.02	0.69	1.78	0.63	0.94	0.52	0.67	0.47	0.46	0.53
16	7.48	1.83	3.95	0.62	1.79	0.59	1.05	0.45	0.62	0.63	0.47	0.58
17	7.04	1.78	3.88	0.48	1.75	0.54	1.14	0.55	0.65	0.65	0.49	0.45
18	6.42	1.83	3.85	0.39	1.91	0.54	1.16	0.37	0.78	0.49	0.49	0.52
19	5.96	1.72	3.76	0.53	1.85	0.57	1.18	0.59	0.79	0.54	0.53	0.57
20	5.52	1.76	3.71	0.32	1.97	0.51	1.27	0.46	0.76	0.45	0.59	0.39

Table A8-12: Means and Standard Deviations (SD) for 3 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.7 Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	6.90	0.90	0.29	0.31	0.02	0.21	-0.04	0.11	0.04	0.10	0.00	0.09
2	9.54	1.25	0.77	0.57	0.12	0.21	0.06	0.25	0.19	0.16	-0.04	0.19
3	11.50	1.45	1.27	0.59	0.19	0.12	0.10	0.20	0.05	0.21	0.00	0.09
4	13.04	1.28	1.94	1.12	0.30	0.28	0.10	0.13	0.11	0.18	0.02	0.19
5	14.30	1.08	2.64	1.52	0.64	0.33	0.24	0.12	0.23	0.13	0.07	0.07
6	15.80	1.43	3.32	1.73	0.84	0.35	0.42	0.27	0.23	0.25	0.16	0.22
7	16.72	1.42	4.19	1.90	1.08	0.52	0.52	0.18	0.38	0.22	0.16	0.23
8	17.74	1.50	4.72	2.14	1.40	0.64	0.64	0.22	0.44	0.23	0.14	0.23
9	18.60	1.68	5.35	2.34	1.56	0.89	0.82	0.31	0.47	0.19	0.16	0.21
10	19.22	2.00	5.95	2.34	1.91	0.91	0.96	0.11	0.50	0.18	0.26	0.16
11	16.12	1.86	5.40	2.14	2.02	0.84	1.03	0.16	0.62	0.26	0.31	0.25
12	14.08	1.81	5.45	2.23	2.14	0.91	1.16	0.18	0.77	0.24	0.31	0.17
13	12.50	2.14	5.64	2.20	2.50	0.85	1.33	0.33	0.89	0.24	0.40	0.28
14	11.40	1.99	5.54	2.21	2.59	0.86	1.46	0.24	0.79	0.21	0.48	0.35
15	10.38	1.95	5.56	2.08	2.68	0.82	1.63	0.37	0.90	0.30	0.46	0.23
16	9.18	1.90	5.45	2.11	2.64	0.87	1.56	0.34	0.96	0.27	0.54	0.37
17	8.54	2.08	5.34	2.06	2.74	0.82	1.66	0.34	0.88	0.30	0.50	0.30
18	7.94	1.96	5.29	1.92	2.81	0.79	1.73	0.32	0.96	0.32	0.55	0.32
19	7.54	2.05	5.04	1.97	2.82	0.74	1.82	0.36	1.12	0.19	0.58	0.46
20	6.96	2.12	4.98	1.85	2.76	0.68	1.86	0.41	1.12	0.31	0.49	0.34

Table A8-13: Means and Standard Deviations (SD) for 3 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 0.9 Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	7.20	0.74	0.54	0.39	0.22	0.24	0.16	0.11	0.11	0.19	0.01	0.08
2	10.56	1.38	1.02	0.91	0.35	0.43	0.14	0.28	0.17	0.16	0.01	0.11
3	13.26	1.67	1.88	1.16	0.52	0.39	0.26	0.19	0.23	0.20	0.07	0.14
4	15.70	1.76	2.76	1.60	0.90	0.57	0.47	0.29	0.34	0.25	0.11	0.12
5	17.22	1.91	3.68	2.03	1.14	0.80	0.47	0.41	0.38	0.33	0.13	0.27
6	18.62	1.87	4.42	2.40	1.66	0.85	0.68	0.56	0.44	0.36	0.29	0.25
7	19.64	2.14	5.21	2.75	1.87	0.96	0.79	0.59	0.49	0.46	0.34	0.32
8	20.74	2.11	6.04	2.99	2.45	1.32	1.08	0.64	0.68	0.59	0.41	0.29
9	21.42	2.21	6.68	3.17	2.82	1.54	1.32	0.94	0.71	0.67	0.46	0.51
10	22.34	2.24	7.42	3.33	3.32	1.76	1.56	1.01	0.92	0.73	0.58	0.47
11	17.92	2.50	7.25	3.22	3.32	1.79	1.74	1.00	1.01	0.79	0.66	0.62
12	15.84	2.29	7.45	3.14	3.48	1.83	1.85	1.23	1.10	0.83	0.64	0.68
13	14.36	1.93	7.43	3.11	3.65	1.74	2.00	1.13	1.18	0.82	0.72	0.58
14	13.14	1.75	7.40	2.85	3.78	1.77	2.10	1.22	1.34	0.88	0.83	0.63
15	12.14	1.79	7.27	2.72	3.96	1.59	2.14	1.13	1.30	0.87	0.79	0.60
16	11.14	1.39	7.14	2.52	3.94	1.55	2.32	1.03	1.45	0.89	0.84	0.61
17	10.70	1.37	6.96	2.37	3.94	1.62	2.48	1.10	1.40	0.85	0.78	0.61
18	9.84	1.32	6.72	2.23	3.96	1.53	2.35	0.91	1.44	0.83	0.83	0.56
19	9.10	1.42	6.59	1.99	3.89	1.37	2.34	1.05	1.43	0.77	0.85	0.60
20	8.44	1.51	6.38	1.81	3.90	1.23	2.45	0.81	1.50	0.78	0.85	0.57

Table A8-14: Means and Standard Deviations (SD) for 3 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 1.1 Watts/cm^2

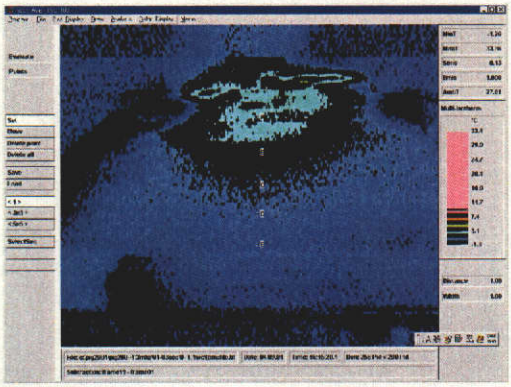
	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	9.38	1.95	0.72	0.53	0.12	0.23	0.05	0.15	0.05	0.11	-0.01	0.11
2	13.36	2.53	1.58	1.04	0.25	0.31	0.14	0.20	0.04	0.08	-0.01	0.13
3	16.44	2.47	2.65	1.58	0.58	0.48	0.31	0.32	0.22	0.25	0.11	0.07
4	19.02	2.59	3.64	1.84	0.89	0.67	0.36	0.34	0.17	0.17	0.08	0.17
5	20.80	1.96	4.76	2.17	1.36	0.91	0.48	0.40	0.24	0.27	0.11	0.20
6	22.46	2.06	5.84	2.29	1.80	1.14	0.66	0.50	0.43	0.25	0.29	0.39
7	23.82	2.11	6.67	2.52	2.32	1.19	1.02	0.45	0.52	0.28	0.23	0.33
8	24.76	1.96	7.63	2.79	2.78	1.36	1.24	0.77	0.66	0.41	0.44	0.34
9	25.74	2.12	8.36	3.21	3.32	1.69	1.52	0.78	0.83	0.40	0.48	0.48
10	26.28	2.07	8.95	3.15	3.65	1.59	1.93	0.96	1.05	0.57	0.61	0.71
11	20.70	1.72	8.41	3.17	3.71	1.51	1.87	0.81	1.01	0.63	0.71	0.58
12	18.28	2.27	8.48	3.16	3.94	1.45	2.19	0.97	1.35	0.62	0.81	0.63
13	16.40	1.92	8.50	3.14	4.14	1.35	2.30	0.80	1.28	0.57	0.74	0.48
14	15.06	2.21	8.40	2.94	4.28	1.31	2.52	0.79	1.35	0.61	0.80	0.60
15	13.64	2.32	8.32	2.70	4.43	1.30	2.78	0.95	1.58	0.56	0.88	0.61
16	12.68	2.04	8.08	2.57	4.44	1.27	2.74	0.83	1.53	0.59	0.82	0.62
17	11.76	2.07	7.95	2.44	4.50	1.22	2.87	0.66	1.62	0.50	0.88	0.52
18	10.92	2.12	7.73	2.22	4.54	1.09	2.96	0.71	1.76	0.70	1.08	0.50
19	10.02	2.03	7.46	2.14	4.55	1.11	3.04	0.62	1.76	0.55	0.95	0.45
20	9.40	1.89	7.24	1.98	4.53	1.04	3.00	0.70	1.74	0.45	1.06	0.46

Table A8-15: Means and Standard Deviations (SD) for 3 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 1.3 Watts/cm^2

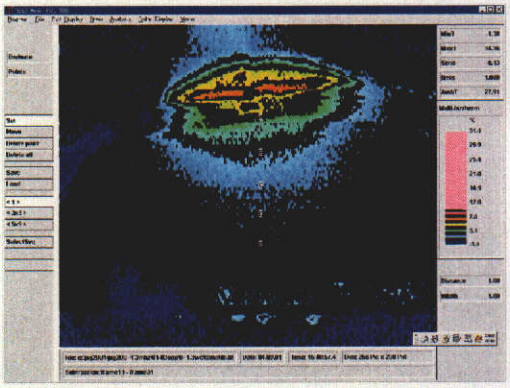
	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	10.56	1.85	0.64	0.32	0.18	0.15	0.10	0.22	-0.01	0.29	0.01	0.21
2	15.16	2.03	1.36	0.78	0.31	0.13	0.11	0.14	0.00	0.34	0.08	0.22
3	18.30	2.89	2.33	1.41	0.37	0.21	0.14	0.21	0.17	0.28	0.13	0.29
4	21.30	2.82	3.43	1.66	0.70	0.26	0.31	0.32	0.17	0.27	0.16	0.23
5	23.32	2.41	4.43	2.06	1.07	0.42	0.30	0.31	0.30	0.40	0.28	0.31
6	24.90	2.88	5.41	2.47	1.49	0.62	0.67	0.40	0.38	0.45	0.29	0.42
7	26.64	2.95	6.19	2.34	1.82	0.70	0.78	0.45	0.58	0.50	0.43	0.58
8	27.42	2.62	6.98	2.77	2.32	0.89	1.18	0.63	0.74	0.61	0.58	0.77
9	28.34	2.57	7.76	2.80	2.72	1.00	1.37	0.75	0.79	0.75	0.66	0.84
10	28.76	2.53	8.57	2.75	3.26	1.21	1.78	0.90	1.01	0.80	0.70	0.95
11	22.94	2.27	7.90	2.40	3.35	1.19	2.04	0.85	1.27	0.83	0.91	1.00
12	19.98	2.33	7.74	2.38	3.68	1.19	2.12	0.88	1.36	0.71	0.95	0.89
13	18.02	2.48	7.82	2.32	3.90	1.17	2.39	0.73	1.54	0.82	1.01	0.84
14	16.30	2.59	7.94	2.17	4.15	1.14	2.66	0.72	1.63	0.67	1.12	0.85
15	14.70	2.40	7.85	2.09	4.24	1.07	2.70	0.71	1.64	0.61	1.18	0.77
16	13.56	2.30	7.70	2.14	4.28	1.04	2.89	0.74	1.79	0.74	1.13	0.84
17	12.82	2.47	7.68	1.83	4.49	0.86	2.96	0.54	1.88	0.68	1.28	0.75
18	11.92	2.39	7.40	1.83	4.42	0.95	2.96	0.67	1.91	0.58	1.25	0.66
19	11.06	2.43	7.21	1.64	4.51	0.79	3.13	0.56	1.99	0.49	1.27	0.65
20	10.36	2.18	7.10	1.62	4.50	0.82	3.12	0.62	2.06	0.58	1.33	0.72

Table A8-16: Means and Standard Deviations (SD) for 3 MHz ultrasound at moderate speed (120 beats/min) for small (2XERA) treatment area at skin's surface and 1, 2, 3, 4, and 5 cm below skin surface at 1.5 Watts/cm^2

	Surface		1cm		2cm		3cm		4cm		5cm	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	10.96	2.16	0.66	0.22	0.18	0.26	0.14	0.18	0.05	0.17	0.05	0.21
2	15.96	2.39	1.27	0.67	0.34	0.25	0.11	0.15	0.11	0.21	0.05	0.34
3	20.34	4.02	2.32	0.86	0.55	0.19	0.26	0.22	0.23	0.25	0.26	0.35
4	22.74	4.11	3.32	0.94	0.82	0.29	0.35	0.28	0.20	0.29	0.10	0.34
5	25.34	4.18	4.32	1.26	1.14	0.28	0.62	0.23	0.40	0.24	0.32	0.30
6	27.04	4.27	5.41	1.06	1.58	0.35	0.92	0.28	0.50	0.28	0.44	0.34
7	28.24	3.92	6.13	1.07	2.24	0.63	1.15	0.40	0.68	0.33	0.60	0.30
8	29.02	3.74	6.86	1.10	2.58	0.64	1.39	0.35	0.77	0.28	0.62	0.43
9	29.90	3.71	7.58	1.02	3.06	0.69	1.74	0.59	1.04	0.39	0.78	0.38
10	30.86	3.95	8.03	1.07	3.54	0.95	2.23	0.62	1.24	0.39	0.80	0.34
11	23.46	1.86	7.61	0.98	3.80	1.02	2.36	0.65	1.45	0.62	1.00	0.43
12	20.38	2.49	7.61	0.95	4.02	1.07	2.58	0.75	1.51	0.57	1.06	0.47
13	18.16	2.13	7.63	1.07	4.27	1.00	2.81	0.90	1.72	0.67	1.09	0.53
14	17.26	2.44	7.61	1.20	4.43	1.04	3.05	0.73	1.69	0.58	1.13	0.60
15	15.86	2.46	7.57	1.14	4.61	1.03	3.14	0.69	1.93	0.62	1.25	0.54
16	14.32	2.35	7.63	1.22	4.81	0.93	3.32	0.78	2.04	0.68	1.43	0.57
17	13.36	1.98	7.46	1.20	4.82	0.92	3.47	0.63	2.10	0.55	1.50	0.46
18	12.50	2.19	7.43	1.24	4.86	0.94	3.58	0.66	2.26	0.64	1.42	0.52
19	11.78	1.87	7.19	1.41	4.80	0.91	3.55	0.71	2.21	0.55	1.49	0.49
20	10.94	1.94	7.02	1.37	4.84	0.88	3.58	0.69	2.29	0.62	1.51	0.54



0.1 Watts/cm^2



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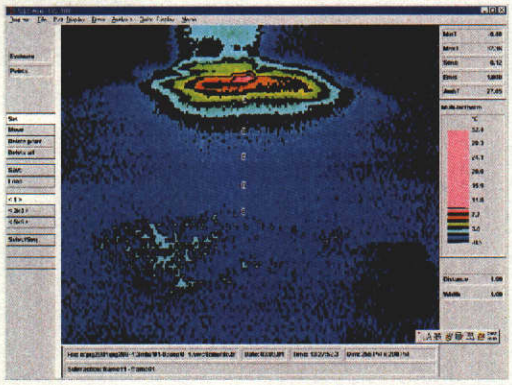

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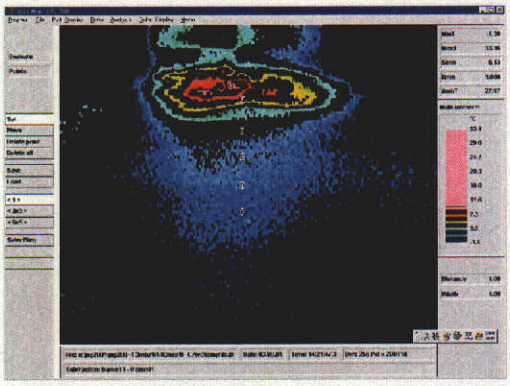

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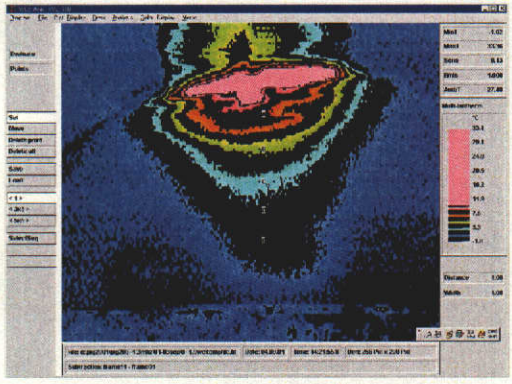

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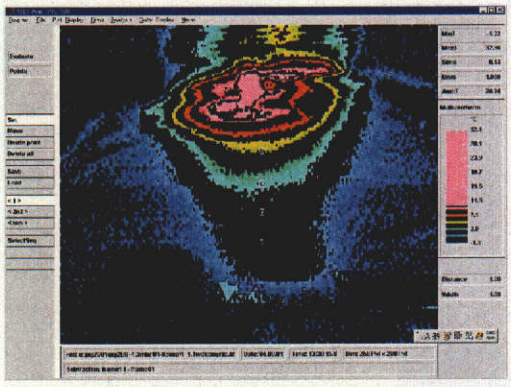
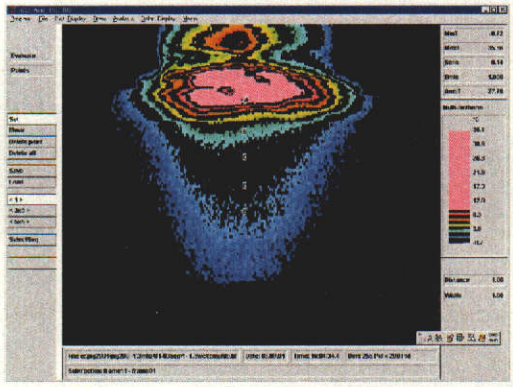
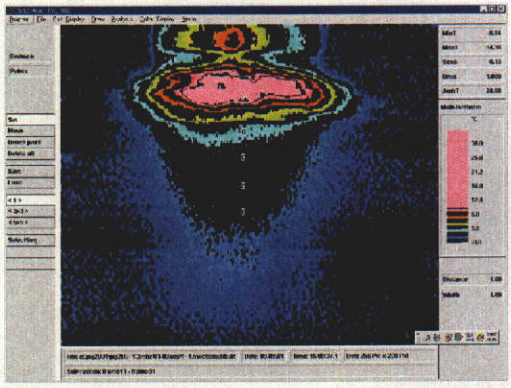


1.1 Watts/cm^2

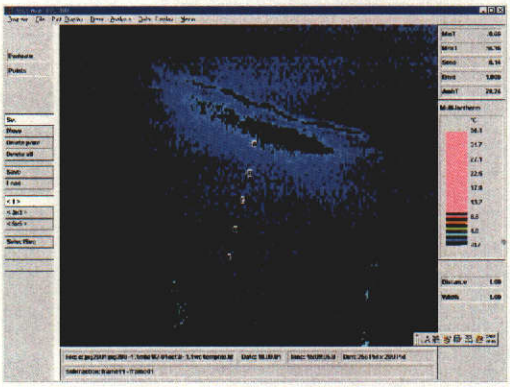


1.3 Watts/cm^2

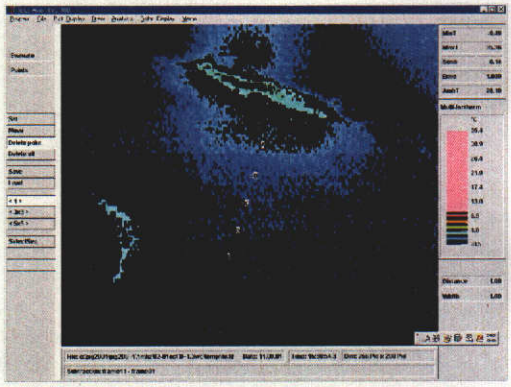


1.5 Watts/cm^2

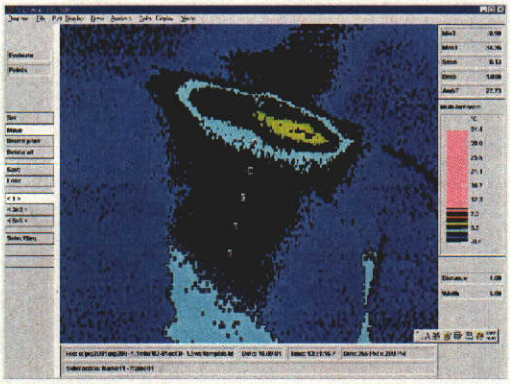
Figure A8-1: Thermographic isothermic scans of various output intensities after 10 minutes exposure to 1 MHz therapeutic ultrasound



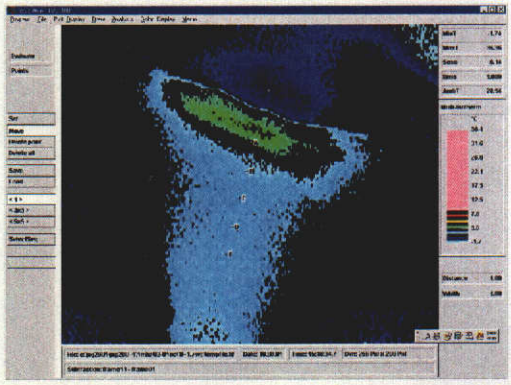
0.1 Watts/cm^2



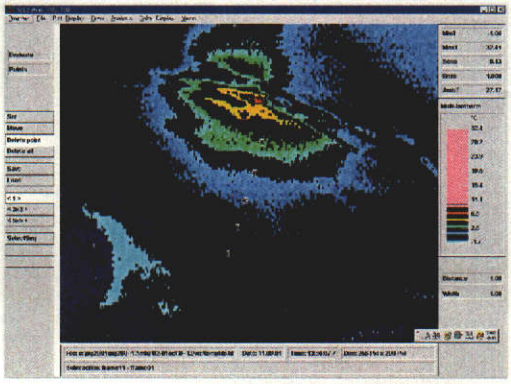
0.3 Watts/cm^2



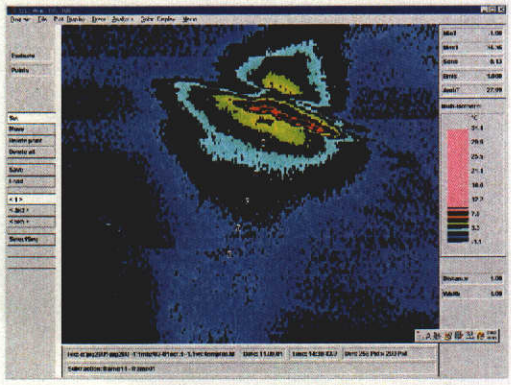
0.5 Watts/cm^2



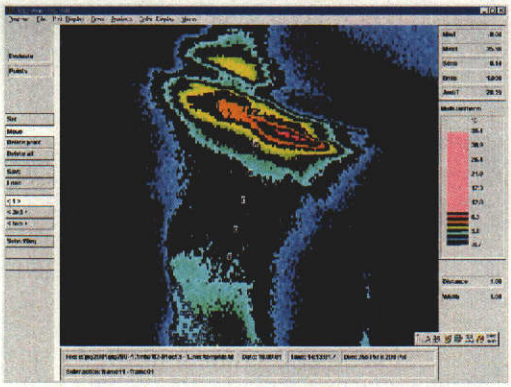
0.7 Watts/cm^2



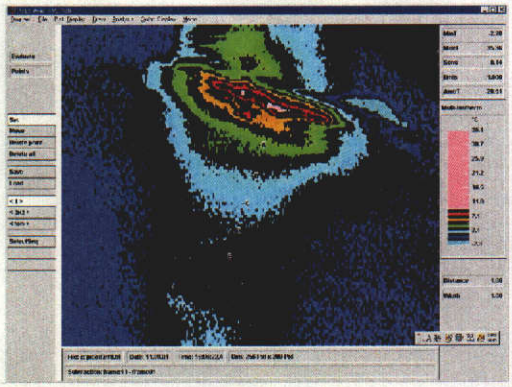
0.9 Watts/cm^2



1.1 Watts/cm^2



1.3 Watts/cm^2



1.5 Watts/cm^2

Figure A8-2: Thermographic isothermic scans of various output intensities after 10 minutes exposure to 3 MHz therapeutic ultrasound