Application of shearwave™ elastography to evaluate heat-induced changes in the young's modulus of fresh bovine muscle: A preliminary study

José Francisco Silva Costa Júnior (jfsc.junior@gmail.com)
Academia da Forca Aerea https://orcid.org/0000-0002-6526-2760

Viviane Bastos Oliveira
Universidade Federal do Rio de Janeiro

Lucas Lobianco De Matheo
Universidade Federal do Rio de Janeiro

Wagner Coelho Pereira
Universidade Federal do Rio de Janeiro

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Abstract

Purpose

The purpose of this study was to investigate the variation in the Young's modulus (E) of bovine muscle samples as a function of temperature change generated by therapeutic ultrasound using Shearwave™ Elastography.

Methods

Initially, the bovine muscle was heated via therapeutic ultrasound with a frequency of 3 MHz, nominal intensity of 2 W·cm\(^{-2}\), and application time of 2 min. Immediately following cessation of therapeutic irradiation, an E image was recorded and the stiffness was measured in circular area positioned at six depths (from 0.4 to 2.9 cm) in the center of the region of interest. Next, an E image was recorded every minute for the first 5 min. Over the next 30 min, an image was recorded every 5 min. Finally, an image was acquired 60 min after cessation of therapeutic irradiation. In the second test, the same experimental procedure was performed 60 min later with the physiotherapy equipment configured with a 10-min application time. Finally, during the ultrasonic irradiation of a new bovine muscle sample, the physiotherapeutic transducer was applied in a circular motion and with an angular velocity of 3.6 ± 0.3 rad·s\(^{-1}\).

Results

In the first test, the bovine muscle E decreased from 212.2 ± 32.8 to 80.1 ± 13.8 kPa at 0.4 cm-depth, as the temperature increased from 18.2 to 44.9 °C. This effect was reversed when the temperature decreased. In the second test, denaturation and cell death occurred, so an artifact appeared in the elastographic image and the Shearwave™ Elastography did not capture the E from the depth of 1.9 cm.

Conclusion

We confirmed that it is possible to use Shearwave™ Elastography to investigate heat-induced changes in the elastic modulus of biological tissue.

Introduction

In physiotherapy, raising the temperature of biological tissues to the range of 40–45 °C for 5 min produces beneficial effects (Guirro and Guirro, 2004), such as pain reduction (Prentice, 2011), so therapeutic ultrasound is typically employed by physiotherapists as a thermotherapy tool. However, if the biological tissue temperature is maintained in the range of 47–50 °C for more than 10 min, protein denaturation may occur (Habash et al. 2006). Based on the same biological effect, membrane rupture and cell shrinkage occur if the temperature is maintained in the range of 60–140 °C for a few seconds. If the temperature is maintained above 50 °C for more than 2 min, cell death may occur (Habash et al. 2006).
The mechanical properties of soft tissues are temperature dependent. Therefore, elastography has excellent potential for evaluating changes in mechanical properties, such as shear modulus (Benech and Negreira 2010; Wu et al. 2001) and strain (Chenot et al. 2010; Kallel et al. 1999; Xia and Thittai 2014), based on variations in tissue temperature, which can be generated through the application of therapeutic ultrasound. One form of therapeutic ultrasound that produces temperature elevation and is widely used as a non-invasive, non-ionizing method to treat a specific known volume (focal region of the ultrasonic transducer) is high-intensity focused ultrasound (HIFU). In this method, high acoustic intensities are applied for a few seconds in the focal region of the transducer, which reduces the occurrence of skin burning, discomfort, and bleeding (Zhou 2014). The acoustic energy is converted into thermal energy and the temperature in the focal region of the transducer is sufficient to destroy tumor structures (Kallel et al 1999; Zhou 2014). Rabkin et al. (2006) showed that 1.15 s after the application of HIFU, the temperature of a rabbit skeletal muscle in vivo increased from 38.5 °C to over 100 °C when an intensity of 490 W·cm⁻² was used. Considering that there will be a temperature gradient for neighboring tissues, it is important to monitor changes in mechanical parameters in the vicinity of the treated area. Elastography has already been employed to evaluate heat-induced changes in soft tissues during HIFU application (Chenot et al. 2010; Kallel et al. 1999; Xia and Thittai 2014; Hynynen 2020). A study by Kallel et al. (1999) revealed that elastography can visualize the treated volume and indicate tissue damage that occurred during treatment.

Large variations in the temperature of biological tissue can cause denaturation of proteins, leading to changes in the shear modulus (μ). Wu et al. (2001) used magnetic resonance elastography (MRE) to study heat-induced changes in the shear modulus of bovine muscle samples at various temperatures during heating and cooling. They noticed that when a region in the tissue reaches high temperature values, changes in the shear modulus become irreversible. Benech et al. (2009) employed time-reversal elastography (TRE) to monitor variations in the μ of a bovine skeletal muscle sample in vitro, which were caused by changes in temperature in the range of 18 – 44.6 °C. They determined that TRE can detect whether or not tissue temperature changes will produce structural changes in the tissue and suggested the use of this technique to monitor the application of HIFU. Benech and Negreira (2010) published a study in which the variation in the shear modulus of a bovine muscle sample in vitro, produced by the heat generated by a 10 Ω electrical resistance positioned inside the sample, was monitored using the 1D transient elastography technique. They were able to detect the critical temperature (between 50 and 60 °C), meaning the temperature at which μ changes irreversibly, and quantify the size of thermal lesions. Liu and Ebbini (2008) also mentioned that the elastographic method can be used to monitor/detect thermal lesion formation.

Non-commercial elastographic equipment based on the supersonic shear imaging (SSI) method has already been used in previous studies (Arnal et al. 2011a; Arnal et al. 2011b; Bercoff et al. 2004; Cortela et al. 2020) to monitor the shear modulus changes due to thermal therapy application in gelatin phantoms and/or ex vivo animal biological tissue samples.
Compared to previous studies, the originality of this work was the application of a commercial elastographic equipment based on the supersonic shear imaging (SSI) method to study the variation in the Young’s modulus (E) at six different depths in bovine muscle as a function of temperature change, which was produced using therapeutic ultrasound equipment. The only ultrasonic elastographic methods previously used to evaluate mechanical property variation based on changes in biological tissue temperature were TRE, 1D transient elastography and non-commercial equipment based on SSI.

Methods

In this study, we used fresh bovine skeletal muscle samples from the same animal sourced from a local butcher shop, so this work was not sent to an Ethics committee or institutional review board.

Supersonic Shear Imaging

The elastographic equipment used in our experiments is based on the SSI technique and is commercially called ShearWave™ Elastography (version 9, Aixplore, Aix-en-Provence, France). It operates sequentially in three modes: imaging, pushing, and imaging. When the device is activated, it initially operates in the imaging mode to acquire a reference image of the medium, then begins to operate in the pushing mode. In this mode, the equipment applies acoustic radiation force by means of beam ultrasonication that is highly focused at three or more different successive depths for a period of approximately 100 µs. In this manner, three or more sources of spherical waves are created, which interact in the form of a Mach cone, creating a quasi-plane wavefront in a 3D conical shape. In the next step, the system returns to operation in the imaging mode and excites the same transducer in bursts with a 5–30-kHz (5,000 to 30,000 images per second) pulse repetition rate to detect the medium vibrations caused by propagation of the shear waves. Initially, the group shear wave speed ($c_s$) is estimated and used to calculate the shear modulus ($\mu$) based on the product of $c_s$ squared and the density (predefined as 1000 kg·m$^{-3}$). Finally, the device presents a map of the $E$ ($E \approx 3\mu$) of the tissue, which is reconstructed by estimating the shear wave speed between two points in the displacement image by means of a time-delay estimation algorithm. The displacement images are obtained by using the cross-correlation technique between the reference image and images acquired after pushing is activated. ShearWave™ Elastography was described in detail by Lima et al. (2018).

Physiotherapeutic Equipment

In our first experiment, E images were recorded from a fresh bovine skeletal muscle sample (13 × 7 × 19 cm), which was heated via physiotherapeutic ultrasound. The transducer was aligned such that its length was parallel to the muscle fibers. Initially, an E image was collected with the physiotherapeutic ultrasound equipment (AVATAR III; KLD Biosisstemas Equipamentos Eletrônicos Ltda, Amparo, SP, Brazil) deactivated. The equipment was then configured to generate a continuous signal with a nominal frequency of 3 MHz, nominal intensity of 2 W·cm$^{-2}$, and application time of 2 min. Immediately following the cessation of physiotherapeutic ultrasound irradiation (in the time of 2 min), ShearWave™
Elastography was used to record an E image, which was saved on the device. Over the first five minutes, an E image was saved every minute. Next, from 7 – 32 min, an elastographic image was saved every five minutes. Thereafter, an image was saved 60 min after the completion of irradiation using the physiotherapy equipment. The temperature of a muscle sample at a distance of 7 cm from the center of the area irradiated by the physiotherapeutic ultrasound equipment ranged from 17.6 – 18.8 °C throughout the experiment and the ambient temperature was 18.8 °C. The transducer with a 3-MHz frequency, which was responsible for irradiating the upper face of the sample, was fixed in a certain position. When irradiation was completed, the therapeutic transducer was removed and the ShearWave™ Elastography transducer was positioned in the same region to evaluate mechanical properties. In addition, the both transducers were coupled to the bovine muscle sample using clinical water-based gel and an acoustic absorber (rubber plate) was placed underneath the muscle sample to avoid unwanted reflections of the ultrasonic wave emitted by the transducer and, thus, prevent the formation of hot spots inside the sample.

A region of interest (ROI) with dimensions of 3.0 × 3.0 cm was selected in all images. In the center of the ROI relative to the x-axis, circular Q-Boxes with a diameter of 5 mm were positioned at the following depths: 0.4, 0.9, 1.4, 1.9, 2.4, and 2.9 cm (Figure 1). ShearWave™ Elastography calculates and displays the mean and standard deviation of the E within each Q-Box, as shown in Figure 1.

In the following experiment, the same fresh bovine skeletal muscle sample was used 60 minutes after the end of the first experiment, but the transducer axis of the physiotherapeutic ultrasound equipment was displaced by 2 cm compared to its previous position along the depth axis. Additionally, the application time for the AVATAR III device was changed to 10 min. Immediately following the cessation of physiotherapeutic ultrasound irradiation, ShearWave™ Elastography was used to record the first E image. E images were then recorded at 10, 13, 18, 20, 25, 30, 35, 40 and 70 min. In this experimental setup, three circular Q-boxes were positioned in the center of the ROI at the following depths: 0.4, 0.9, and 1.4 cm.

In the final experiment, during ultrasonic irradiation, the transducer coupled to the physiotherapeutic equipment was applied for 10 min in an area corresponding to 5.5-ERA (ratio between the irradiated area and the ERA, effective radiating area) in a fresh bovine skeletal muscle sample in a circular motion and with an angular velocity of $3.54 \pm 0.30 \text{ rad}\cdot\text{s}^{-1}$ (33.8 ± 2.82 rpm), which was measured 10 times by counting the number of revolutions per minute of the transducer inside an acrylic ring with internal radius of 2.68 ± 0.01 cm. When the physiotherapeutic equipment operates at 3 MHz, the ERA is 4.09 cm². Immediately following the cessation of ultrasound irradiation (in 10 minutes), ShearWave™ Elastography was employed to record an E image. Over the first five minutes, an E image was captured every minute. From 15–40 min, an E image was saved every five minutes. Thereafter, an image was saved at 70, 100, and 130 min after the completion of irradiation with the physiotherapy equipment. Six circular Q-boxes with a diameter of 5 mm were positioned at the following depths: 0.4, 0.9, 1.4, 1.9, 2.4 and 2.9 cm.

Figure 2 summarizes the parameters (nominal frequency, nominal intensity, application time and transducer movement) used in the therapeutic ultrasound device, which was used to heat the bovine...
muscle. In addition, the instant of time that the elastographic images were acquired in each experiment were presented.

Infrared Camera

Two bovine muscle samples (7.0 × 3.0 × 12.0 cm) connected at one end by a thin layer of fat (< 0.3 mm) were used to study the maximum temperature and thermal field generated by physiotherapeutic ultrasound inside the samples with the same experimental configuration as that used for the SSI experiments, for tests 1 (application time of 2 min) and 2 (application time of 10 min and with transducer in a fixed position). These samples were placed parallel and in contact to each other, forming a unique sample with dimensions of 7.0 × 6.0 × 12.0 cm. Additionally, the physiotherapeutic transducer was positioned in the same position (top surface of the sample) as the transducer coupled to the ShearWave™ Elastography device that captured E images. An infrared camera lens (E6, FLIR® Systems Inc, Boston, MA, USA) was positioned parallel to the upper surface of the fresh bovine skeletal muscle sample, at a distance of 50 cm and the ambient temperature was 23.3 °C. Initially, the physiotherapeutic transducer was coupled to the upper face of the bovine muscle sample using clinical water-based gel and the muscle sample was placed on an acoustic absorber (rubber plate). So, the physiotherapeutic device was configured in a similar way to the first test performed with SSI, then the tissue irradiation was started. Immediately after the physiotherapeutic equipment completed ultrasonic irradiation, the bottom faces of the two samples (connected at one end by a thin layer of fat) were placed in contact and a thermal image was recorded by the infrared camera. This process took approximately 10 s (see Figure 3). In the static situation, the axis of symmetry of the ultrasonic transducer was positioned at the top between the two samples. In the dynamic situation, the center of the treatment area was positioned at the top between the two samples. Finally, the same procedure described above was performed with the physiotherapeutic ultrasound equipment configured in a similar way to test 2.

The thermal images also showed the thermal field inside the tissue, which can be related to information regarding E as a function of depth. These thermal images were obtained considering the configurations of the physiotherapeutic device used in the two tests performed with ShearWave™ Elastography device. In order to confirm the temperature value obtained with the infrared camera, the values obtained with this device were compared with the temperature values recorded by a digital thermometer (Model 52, Fluke, Everett, WA, USA). An ultrathermostat bath (524-2D, Nova Ética, São Paulo, SP, Brazil) was used to facilitate comparison of the temperature values obtained by the two devices. For this purpose, the ultrathermostat bath was set to maintain the temperature of its reservoir at values of 25, 30, 35, 40, 45, 50, 55, and 70 °C, and the water temperature was recorded five times for each thermal device. The temperature values measured with the infrared camera (\(T_{IC}\)) and the digital thermometer (\(T_{DT}\)) were applied in \(\text{error} = \frac{(T_{IC} - T_{DT})}{T_{DT}} \cdot 100\) to estimate the relative error of each measurement. Finally, the mean and standard deviation of the relative error will be calculated.

Results
ShearWave™ Elastography

The mean and standard deviation of E estimated by the ShearWave™ Elastography device are presented in Figure 4 as a function of time for an application time of 2 min. The first point (mean and standard deviation values of E) presented in this figure (0 min) was obtained with the physiotherapeutic equipment turned off, regardless of the depth analyzed. The second point was obtained immediately after the physiotherapeutic equipment finished ultrasonic irradiation (immediately 2 min after the start of irradiation).

Figure 5 shows the mean and standard deviation of E as a function of time for an application time of 10 min. In all depths, the first two points (mean and standard deviation values of E) were obtained with the physiotherapeutic equipment turned off (0 min) and immediately after the physiotherapeutic equipment finished ultrasonic irradiation (10 min), respectively.

Figure 6a presents the B-mode (left) and E (right) images captured before the physiotherapy ultrasound equipment was activated. The other images were recorded 10 min after the end of ultrasonic irradiation with application times of 2 and 10 min (Figures 5b and 5c, respectively). Red arrows in the B-mode images represent an increase in the hypoechoic region, which indicates changes in the bovine muscle structure. The yellow arrow in Figure 6c represents the region where tissue contraction occurred compared to the region indicated by the yellow arrow in Figure 6a. Additionally, a comparison of the regions indicated by the white arrows in Figures 6b and 6c reveals that there was a drastic change in the elastographic image (there is a region of negative color fill in Figure 6c), indicating an irreversible change in the mechanical properties of the tissue.

Following therapeutic irradiation with the transducer in a static position on the sample and an application time of 10 min, a permanent alteration of the structure of the biological tissue was observed (Figure 7). It should be noted that 30 and 60 min after irradiation stopped, structural damage can still be visualized in both the B-mode and E images (Figures 7b and 7c). The red arrows indicate the regions in the B-mode images that showed increased tissue damage. The white arrows in Figure 7a, 7b, and 7c indicate increases in the E of the bovine tissue.

The mean and standard deviation of E as a function of time for the physiotherapeutic transducer with circular motion and an application time of 10 min are presented in Figure 8.

Infrared Camera

Table 1 lists the reference temperature (RT) values that were obtained by the ultrathermostat bath. Additionally, the table shows the mean and standard deviation of the temperature values obtained by the digital thermometer and infrared camera, as well as the mean and standard deviation of relative error (%) for the two measurement types. In this case, the temperature values obtained by the thermometer were used as reference values. Additionally, the difference between the highest temperature recorded inside the bovine sample by the infrared camera and digital thermometer was less than 2.5 %.
Table 1. Reference temperature (RT) mean, and standard deviation of temperature values obtained by digital thermometer and infrared camera, as well as the mean and standard deviation of relative error (%) for the two measurement types

| RT (°C) | Infrared Camera | Digital Thermometer | Relative Error (%) |
|--------|-----------------|---------------------|-------------------|
| 25     | 24.96 ± 0.46    | 25.04 ± 0.05        | -0.32 ± 1.84      |
| 30     | 30.16 ± 0.39    | 29.46 ± 0.05        | 2.38 ± 1.34       |
| 35     | 34.84 ± 0.05    | 34.02 ± 0.08        | 2.41 ± 0.33       |
| 40     | 40.42 ± 0.54    | 39.36 ± 0.05        | 2.69 ± 1.25       |
| 45     | 44.58 ± 0.08    | 43.84 ± 0.11        | 1.69 ± 0.13       |
| 50     | 48.78 ± 0.16    | 48.66 ± 0.05        | 0.25 ± 0.30       |
| 55     | 53.46 ± 0.11    | 53.46 ± 0.05        | 0.00 ± 0.26       |
| 60     | 57.84 ± 0.11    | 58.00 ± 0.07        | -0.28 ± 0.15      |
| 65     | 63.36 ± 0.35    | 63.44 ± 0.05        | -0.13 ± 0.49      |
| 70     | 69.20 ± 0.39    | 68.46 ± 0.11        | 1.08 ± 0.49       |

The thermal fields generated by the application of physiotherapeutic ultrasound to a bovine muscle sample with application times of 2 and 10 min are visualized in Figure 9. The white regions in Figures 9a and 9b represent temperatures above 40 and 50 °C, respectively. The FLIR® software was used to obtain the maximum, minimum, and average temperatures inside the circles in the white regions. The circular areas in Figure 9 are 5.31 and 10.2 cm² (radius of 1.3 and 1.8 cm) and the average temperature in these areas were 41.2 and 60.2 °C, respectively. When the circular motion is done there is a reduction of 11% in the average temperature found into the thermal field compared to the 10min static mode.

Figure 10 presents photos of the samples of fresh bovine skeletal muscle before (top) and after (bottom) irradiation with the ultrasound equipment with an application time of 10 min. This figure shows denaturation and cell death, as indicated by the red arrows (bottom). The region that was damaged by acoustic irradiation is indicated by green arrows on the tissue before irradiation (top). One can see that there were no burns prior to irradiation.

**Discussion**

ShearWave™ Elastography is a powerful tool to analyzing tissue stiffness. Over the years, many elastography technical possibilities were found to be relevant on this subject (Benech and Negreira 2010;
Hynynen 2020; Liu and Ebbini 2008). In this study, we revealed that it is possible to apply an commercial elastographic equipment based on the supersonic shear imaging (SSI) to investigate heat-induced changes of the E of biological tissue, collaborating and adding novelty to the work of other groups.

Benech et al. (2009) mentioned that the effects of heating on time shift are negligible when compared to shear wave speed variation and E variation. In addition, the ShearWave™ Elastography disregarded temperature effects on the radiofrequency signals used in the calculation of time delay, which is used to estimate the group shear wave speed.

A study by Wu et al. (2001) revealed that bovine muscle shear modulus decreases with increasing temperature, but this process can be reversed when the temperature decreases if the maximum temperature was less than 55 °C. The same effect was observed in this study at all six depths for the physiotherapeutic transducer at rest and with an application time of 2 min, which resulted in a maximum temperature of 44.9 °C (See Figure 4). The slight difference between the reference value of E and the value of E 60 min after the end of acoustic irradiation may be a consequence of the small temperature difference between the beginning and end of the experiment (1.2 °C).

In Figures 5 and 6, can be seen that the elastographic equipment based on the SSI method was not able to calculate E after the denaturation of cells in a given region, which drastically increased the stiffness of the biological tissue. The regions with negative color filling in the E images in Figures 6 and 7 correspond to the damaged regions and represent a type of artifact in the ShearWave™ Elastography system. Lin et al. (2017) mentioned that it is possible that artifact occurrence may be related to the stiffness value of the studied medium. They conducted a study on image artifacts generated by SSI using an acrylic plate and bean curd. They found that artifacts appeared in the superficial layer of the acrylic plate, but did not occur in the bean curd. According to Deffieux et al. (2011), artifact origins in elastographic images may be related to the boundary effects produced by the reconstruction of two-plane shear waves, which is calculated based on the coherent sum of shear waves generated at three or more different successive depths. Some possible mechanisms of artifact generation can be visualized in the works by Deffieux et al. (2011) and Lin et al. (2017).

A comparison of the B-mode images in Figures 6a, 6b, and 6c reveals that in Figure 6c, there was a structural change in the tissue, especially in the upper portion, where the transducer was positioned. There is a shadow below the region that presented structural changes, which could not be displayed in the E images, which implies a limitation of the equipment based on SSI because the equipment based on MRE (Wu et al. 2001) successfully captured the mechanical properties of both the thermally damaged tissue and neighboring regions. Additionally, devices based on harmonic motion imaging (Maleke and Konofagou 2008) and 1D transient elastography (Benech and Negreira 2010) are able to assess structural changes in tissue as a function of depth. Figure 10 presents samples of the bovine muscle before (preserved cells) and after (denaturation and cell death) physiotherapeutic irradiation with the ultrasound equipment configured with an application time of 10 min and the transducer at rest. Additionally, it was not possible to use the elastography equipment to record the E during
physiotherapeutic irradiation (with the transducer axes parallel or perpendicular) because there was acoustic interference, which did not allow for the elastographic mode to operate.

When tissue temperature exceeds 70 °C, the cells shrink as much as possible, meaning most of the water in the cells is released (denaturation of the cells) through tissue coagulation (Wiederhorn and Reardon 1952). Additionally, Kiss et al. (2009) mentioned that increases in the magnitude of the complex modulus result of tissue contraction and the release of water from cells, which explains the effects observed in Figure 6c.

In Figure 8, at the first four depths, it was possible to observe an increase in the $E$ of the tissue as a function of time with an application time of 10 min and circular transducer movement. Wu et al. (2001) heated a bovine tissue sample from 20 °C to approximately 75 °C, then cooled it back to room temperature. When the muscle temperature ranged from 60–75 °C (Region II), there was an almost linear increase in the shear modulus, meaning the $\mu$ value was greater than the shear modulus of the sample prior to heating. At depths five and six, there was a reduction of approximately 51% in the value of $E$ measured immediately upon the cessation of irradiation. However, there was an increase in $E$ of approximately 90% between the times of 30 s and 120 min. Despite this increase, the values of $E$ estimated 120 min after the end of acoustic irradiation were 68.5% and 69.6% less than the reference $E$ for depths five and six, respectively. According to a study presented by Benech and Negreira (2010), and Wu et al. (2001), these differences in the value of $E$ indicate that the tissue reached a critical temperature and the change in $E$ became irreversible.

Figure 9a illustrates an interesting effect because the temperature was maintained in the range recommended by physiotherapists, but the parameters used for the physiotherapeutic equipment are not used in clinical practice. Specifically, the transducer was fixed in a particular position during ultrasonic irradiation. However, it was shown in Figure 4 that there was a change in $E$ of over $-150$ kPa based on the temperature variation from 18.2 – 40.8 °C. In a study by Wu et al. (2001), a sample of bovine muscle was heated from 20 °C to approximately 55 °C. They also observed a change in $E$ of approximately $-150$ kPa. In this situation, when the sample is cooled (back to the initial temperature), the mechanical properties of the tissue return values similar to the initial values (before heating). According to Wu et al. (2001), reversible protein denaturation may be an acceptable explanation for the changes in the mechanical properties of biological tissue in heating ranges below 55 °C.

This study showed that commercial equipment ShearWave™ Elastography can be used to evaluate *in vivo* the effect of therapeutic ultrasound on the stiffness of human tissue, because the temperature range devised by the physiotherapists (40 – 45 °C) generates a reversible change in the stiffness of the biological tissue.

Figure 9b indicates that the temperature is high in areas close to the physiotherapeutic transducer at a depth of less than 3.5 cm (half of the sample). However, thermal conduction promotes a reduction in temperature in this area and an increase in temperature in the surrounding areas. This effect is
associated with the low thermal conductivity (0.52 W·m⁻¹·K⁻¹ for muscle) of the sample and the long duration for which these regions are subjected to high temperatures, which can lead to tissue damage, as revealed by comparing the B-mode images in Figures 7a, 7b, and 7c. Habash et al. (2006) mentioned that protein denaturation, membrane rupture, cell shrinkage, and/or cell death may occur when the temperature is maintained above 50 °C for tens of seconds.

Although the Q-box of the elastographic image has an area of only 0.79 cm², there is a high difference between the maximum and minimum temperature values inside the Q-box positioned at the depth of 0.4 cm, when compared to the Q-boxes positioned at other depths. The propagation of the shear wave in a medium with constant temperature makes the measurement of stiffness more accurate, reducing the stiffness standard deviation. Therefore, the standard deviation in the first Q-box is greater than the standard deviation of the other Q-boxes with a more homogeneous temperature distribution (see Figures 4 and 8). This analysis can be performed from the thermal field shown in Figure 9. In Figure 9b, the difference between the maximum and minimum temperature in the Q-boxes is very close, so the standard deviation is practically the same magnitude in all depths. In addition, the standard deviation of Young's modulus shown in Figure 8 is greater than the standard deviation of E shown in Figure 4, which occurs due to the generation of the critical temperature in Test 2. This temperature causes protein denaturation and cell death. It is worth mentioning that these consequences are part of a dynamic process because of the temperature gradient and occur while the sample stiffness is measured. In Figure 5, in the region with a temperature equal to or above 70 °C, protein denaturation and cell death has occurred and for that reason, it was not possible to measure the stiffness of the tissue because an image artifact has been formed (see Figure 7). Due to thermal conduction and high temperature, the spread of heat to depths 1 and 2 increased the standard deviation and reduced the stiffness difference over time. In addition, cell death may not have occurred at these depths, but as it can be seen in Figure 10, cell denaturation has occurred.

The results in Table 1 indicate that the thermal values obtained via infrared camera are reliable because the largest relative error was less than 2.5 % when using the values obtained by the digital thermometer are reference values. In addition, when the infrared camera registered maximum temperatures of 44.9 and 70.2 °C inside the bovine sample, the difference between those temperatures and the ones recorded by the digital thermometer was less than 2.5 %.

ShearWave ™ Elastography is a powerful tool to help physiotherapists evaluate the quality of the treatment by analyzing tissue stiffness, but the physicians can not use this commercial equipment to identify lesions. This study revealed that it is possible to apply elastographic methods based on the SSI technique to investigate heat-induced changes of the E of biological tissue. In situation where irreversible changed in the E occurred, an image artifact (negative color fill) was formed based on protein denaturation. Such artifacts indicate that this method cannot be employed to monitor the use of HIFU. These regions of negative color fill in elastographic images (artifacts) should be investigated in detail in the future.
There are some limitations in this study. First, it was not possible to generate elastographic images during ultrasonic irradiation with the physiotherapy equipment based on the interference of the waves in this equipment with the elastography equipment, even when the central axes of the transducers of the two devices were perpendicular to each other. This made it impossible to evaluate the reduction in stiffness with increasing temperature. Finally, the three experiments were performed only once.

**Conclusion**

In the present study, the commercial elastographic equipment based on the supersonic shear imaging (SSI) method (Shearwave™ Elastography) was used to investigate the variation in the Young's modulus (E) at six different depths in bovine muscle as a function of temperature change, which was produced using therapeutic ultrasound. It is possible to apply Shearwave™ Elastography (commercial elastographic equipment) to investigate heat-induced changes of the E of biological tissue. Additionally, this method cannot be employed to monitor the use of HIFU because of the image artifacts that are produced by the rise in temperature, which results in cell death and protein denaturation. It is important that more studies are carried out to explore the limitations of this study.

**Declarations**

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**Conflict of Interest**

The authors declare that there are no conflicts of interest.

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Figures

Figure 1

Illustration of ROI markup in the B-mode image (left) and circular Q-Boxes with a diameter of 5 mm positioned at depths of 0.4, 0.9, 1.4, 1.9, 2.4 and 2.9 cm in the center of the ROI. The elastographic mode (right) displays the E in the ROI.
Figure 2

Summary of the parameters used in the physiotherapeutic device and the times utilized to record E images in the ShearWave™ Elastography.
Figure 3

Scheme describing the procedures for the recording thermal images of the interior of the tissue. a. Two samples were placed parallel and in contact with each other. b. The physiotherapeutic transducer was positioned on the top surface of the samples. c. Immediately after the physiotherapeutic equipment finished ultrasonic irradiation, the bottom faces of the two samples were placed in contact. d. A thermal image was recorded by the infrared camera. e. Thermal image captured by the infrared camera.
Figure 4

Mean and standard deviation of $E$ estimated as a function of time for the physiotherapeutic transducer at rest, with an application time of 2 min and immediately following the cessation of ultrasound irradiation.
Figure 5

Mean and standard deviation of $E$ estimated as a function of time for the physiotherapeutic transducer at rest, with an application time of 10 min, immediately following the cessation of ultrasound irradiation and with 60 min after the first test.
Figure 6

B-mode images (left) and corresponding E images (right). a. Images captured before the physiotherapy ultrasound equipment was activated. Images (b. and c.) recorded 10 min after the cessation of ultrasonic irradiation with application times of 2 and 10 min, respectively. Red arrows in the ultrasound images represent an increase in the hypoechoic region, indicating changes in tissue structure. Yellow arrows indicate tissue contraction and the white arrows indicate a drastic change in the elastographic images.
Figure 7

B-mode images (left) and corresponding E images (right). The physiotherapy ultrasound equipment was configured with application time of 10 min and images were recorded a. 2 min b. 30 min, and c. 60 min after the cessation of ultrasonic irradiation. White arrows indicate increased tissue stiffness and red arrows indicate increases in the hypoechoic region in the ultrasound images.
Figure 8

Mean and standard deviation of $E$ as a function of time for the physiotherapeutic transducer with circular motion, an application time of 10 min and immediately following the cessation of ultrasound irradiation.
Figure 9

Thermal field generated by physiotherapeutic ultrasound with application times of a. 2 min and b. 10 min. The maximum, minimum, and average temperatures in the circular regions in the thermal images are displayed.

Figure 10

Samples of bovine muscle before (top) and after (bottom) irradiation with the ultrasound equipment configured with an application time of 10 min.