http://dx.doi.org/10.1590/1678-6676

***Arq. Bras. Med. Vet. Zootec.*, v.66, n.5, p.1343-1350, 2014**

Heating produced by therapeutic ultrasound in the presence of a metal plate in the femur of canine cadavers

[*Aquecimento gerado pelo ultrassom terapêutico em presença de placa óssea metálica no fêmur de cadáveres caninos*]

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ABSTRACT

The present study aimed to assess the heat generated by a therapeutic ultrasound (TUS) in a metal bone plate and adjacent structures after fixation to the femur of canine cadavers. Ten pairs of hind limbs were used, and they were equally distributed between groups that were subjected to 1- and 3-MHz frequencies, with each frequency testing 1- and 2-W/cm² intensities. The right hind limb was defined as the control group (absence of the metal plate), and the left hind limb was the test group (presence of the metal plate). Therefore, the control groups (CG) were denominated CGI, using TUS with 1-MHz frequency and 1-W/cm² intensity; CGII, using 1-MHz frequency and 2-W/cm² intensity; CGIII, using 3-MHz frequency and 1-W/cm² intensity; and CGIV, using 3-MHz frequency and 2-W/cm² intensity. For each control group, its respective test group (TG) was denominated TGI, TGII, TGIII and TGIV. The TUS was applied to the lateral aspect of the thigh using the continuous mode and a 3.5-cm² transducer in a 6.25-cm² area for 2 minutes. Sensors were coupled to digital thermometers that measured the temperature in different sites before (t_0) and after (t_1) of the TUS application. The temperatures in t_1 were higher in all tested groups. The intramuscular temperature was significantly higher ($P < 0.05$) in the groups used to test the 3-MHz frequency in the presence of the metal plate. The therapeutic ultrasound in the continuous mode using frequencies of 1 and 3 MHz and intensities of 1 and 2 W/cm^2 for 2 minutes caused heating of the metal plate and adjacent structures after fixation to the femur of canine cadavers.

Keywords: dog, physical therapy, deep heat therapy, heating, contracture

RESUMO

O objetivo deste estudo foi avaliar o aquecimento gerado pelo ultrassom terapêutico (UST) na placa óssea metálica e estruturas adjacentes após a fixação no fêmur de cadáveres caninos. Foram utilizados dez pares de membros pélvicos, distribuídos igualmente entre os grupos que utilizaram as frequências de 1 e 3 MHz. Cada frequência testou as intensidades de 1 e 2 W/cm², sendo que o membro pélvico direito foi definido grupo controle (ausência da placa óssea metálica) e o membro pélvico esquerdo o grupo teste (presença da placa óssea metálica). Portanto, os grupos controles foram denominados GCI, com UST na frequência de 1 MHz e intensidade de 1 W/cm²; GCII, com 1 MHz e 2 W/cm²; GCIII, com frequência de 3 MHz e intensidade de 1 W/cm²; e GCIV, com 3 MHz e 2 W/cm². Para cada grupo controle, seu respectivo grupo teste foi denominado GTI, GTII, GTIII e GTIV. O UST foi aplicado na face lateral da coxa, utilizando o modo contínuo, transdutor de 3,5cm², em uma área de 6,25cm², durante dois minutos. Foram utilizados sensores acoplados a termômetros digitais que mediram a temperatura em diferentes locais antes (t_0) e após (t_1) a aplicação do UST. Pode-se verificar que as temperaturas em t_1 foram maiores em todos os grupos testados. Os grupos que testaram a frequência de 3 MHz demonstraram que a temperatura intramuscular foi significativamente maior ($P<0,05$) na presença da placa óssea metálica. O ultrassom terapêutico no modo contínuo de 1 e 3 MHz e intensidades de 1 e 2 W/cm^2 durante dois minutos promove o aquecimento da placa óssea metálica e estruturas adjacentes após a fixação no fêmur de cadáveres caninos.

Palavras-chave: cão, fisioterapia, termoterapia profunda, aquecimento, contratura

Recebido em 21 de março de 2013
Aceito em 11 de fevereiro de 2014

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INTRODUCTION

One of the complications observed after femoral fractures in dogs and cats is the quadriceps contracture, which causes fibrosis and adhesion of this muscle group to the developing callus, resulting in ankylosis and hyperextension of the knee joint (Davidson *et al*., 2005). Fibrotic myopathy also occurs in the gracilis, semimembranosus and semitendinosus muscles and in the biceps femoris. Fibrotic myopathy is the replacement of muscle fibers by fibrous connective tissues, causing the flexion contracture of the muscles involved (Doyle, 2004). Trauma, pain and post-operative edema of femoral fractures can be causes of these disorders (Steiss, 2002). These complications can be avoided or treated with immediate post-operative rehabilitation using specific techniques of muscle and joint mobilization. To increase the efficiency of these techniques, prior heating of the structures involved using deep-heating agents, such as therapeutic ultrasound, is recommended (Davidson *et al*., 2005).

Therapeutic ultrasound (TUS) is within the acoustic spectrum and uses high-frequency sound vibrations. The frequencies of 1 and 3 MHz are used most often and determine the wave penetration at depths ranging from 2 to 5cm and 0.5 to 3cm, respectively (O'Brien Junior, 2007; Watson, 2008). The intensity refers to the rate of energy released per area unit and generally ranges from 0.25 to 3 W/cm². The effects of the TUS are identified as non-thermal and thermal according to the sound wave release, which can be pulsed or continuous (Warden *et al*., 2002; Steiss *et al*., 2004).

The continuous mode TUS promotes tissue heating and a consequent increase in collagen extensibility, local blood flow, nerve conduction velocity and pain threshold, which reduce muscle spasm, contracture and adhesion (Baker *et al*., 2001; Canapp, 2007. However, its use in areas with metal implants is still controversial, with some authors stating that there are no problems (Lehmann *et al*., 1959), whereas others recommend precaution (Steiss *et al*., 2004) and even contraindicate it (Young, 2003). In the presence of a metal implant, TUS application could cause a local overheating with serious damage to the adjacent tissues (Gersten, 1958).

The tissue heating generated by the TUS have been studied in different experimental models, as human's tendon and muscle (Draper *et al*., 1995), human's cadaver muscle (Cambier *et al*., 2001), rats (Okita *et al*., 2009) and dog's muscle (Levine *et al*., 2001), dog's muscle and bones (Herrick, 1953) and pig's cadavers muscle (Demmink *et al*., 2003). Others studies were carried out to evaluate the temperature and the TUS effects in the presence of metallic implant applied the metal bone´s plate in pigs (Lehmann *et al*., 1958; Lehmann *et al*., 1959), in dogs (Skoubo-Kristensen *et al*., 1982), in rabbits (Garavello *et al*., 1997), and in addition to intramedullary pin in rat's femur (Kocaoğlu *et al*., 2011).

Considering the subject relevance, the lack of experimental studies and the lack of consensus on the use of TUS in the presence of a metal implant, the present study aimed to assess the heating produced by TUS in the continuous mode at 1- and 3-MHz frequencies and at 1- and 2-W/cm² intensities on the metal plate and adjacent structures after fixation to the femur of canine cadavers.

MATERIAL AND METHODS

Ten pairs of hind limbs from cadavers of adult mongrel dogs were selected from the Institution's Laboratory of Veterinary Pathology. The samples were numbered and subjected to trichotomy, plain radiographs of the femur and thigh perimetry to identify changes or asymmetries that would exclude them from the experiment. Then, the midpoint between the greater trochanter and the lateral condyle was located to measure the distance between the skin and the femur with an 18-G catheter and a caliper, and regions with a thickness between 1 and 3cm were selected. The samples were then frozen at -36°C. Prior to use, they were thawed in a climate-controlled room at 18°C±1°C for at least 16 hours.

The ten pairs of hind limbs were equally distributed among the groups that used the 1- and 3-MHz frequencies. Each frequency tested the 1- and 2-W/cm² intensities. The right hind limb was defined as the control group (CG), where the TUS was applied in the absence of a metal plate, and the left hind limb was defined as the test group (TG), with the TUS being applied in the presence of a metal plate fixed to the femur. Therefore, the control groups were denominated CGI, using a frequency of 1 MHz and an intensity of 1 W/cm²; CGII, using 1 MHz and 2 W/cm²; CGIII, using 3 MHz and 1 W/cm²; and CGIV, using 3 MHz and 2 W/cm². The respective test group of each control group was denominated TGI, TGII, TGIII and TGIV.

The TUS (Ibramed Sonopulse Diamont Line of 1 and 3 MHz; Brazilian Industry of Medical Equipment, Ltd., Brazil) was used in all of the experimental groups in the continuous mode, using a transducer with a 3.5-cm² effective radiating area (ERA) in a 6.25-cm² area (A) for 2 minutes (T) (T = A/ERA) and a transducer movement speed of 2cm/s. The skin area for the TUS application was delimited by the lateral aspect of the thigh involving the vastus lateralis muscle, and the center of this area corresponded to the center of the metal plate. The area was covered with a 0.5-cm layer of water-based gel as a coupling medium at the moment of application.

In the test groups (TGI, TGII, TGIII and TGIV), the metal implant placement was performed by exposing the femoral diaphysis via a lateral approach, according to the technique described by Piermattei *et al*. (2006). The 3.5-mm 316L stainless steel metal plate (ORTOVET; Commercial Veterinary Orthopedics, Ltd., Brazil) with ten holes was shaped to the femur and fixed between the greater trochanter and the lateral condyle by eight screws with varying length, leaving the two holes in the center of the plate free.

Four thermocouple sensors coupled to two portable digital thermometers (Instrutherm Model TH-095; Instrutherm Measuring Instruments, Ltd., Brazil) and three thermocouple sensors (Digital Thermometer Model SH-113; Suzhou Jingle Electronics Technology Co., Ltd., China) were used to measure the temperatures. The thermocouple sensors (S) coupled to the portable digital thermometers were denominated S1, S2, S3 and S4 and were fixed to the medial side of the femur through holes in the cortical bone made with a 3.0-mm orthopedic drill coupled to a drilling machine (ORTOVET; Commercial Veterinary Orthopedics, Ltd., Brazil). The S1 and S3 sensors were placed in the medullary cavity, and the S2 and S4 sensors were in contact with the inner lateral cortical bone of the femur (CGI, CGII, CGIII and CGIV) or with the metal plate (TGI, TGII, TGIII and TGIV). The S2 sensor was contralateral to the center of the plate, and the S4 sensor was in the interval between the two distal screws of the plate (Figure 1A and 1B). The S1 and S3 sensors were set at a 1.2-cm distance, cranial to S2 and S4, respectively.

On the lateral side of the limb, three thermocouple sensors (S) were used, and they were located at different points denominated S5, S6, S7 and S8. The sensor that measured the S5 temperature remained in contact with the lateral cortical bone of the femur (CGI, CGII, CGIII and CGIV) or the metal plate (TGI, TGII, TGIII and TGIV), and after measuring the temperature, it was retracted by 0.5cm to measure the intramuscular temperature, denominated S6. This sensor was located in the center of the TUS incidence area; therefore, it was removed at the moment of the application. The S7 and S8 sensors were included only in the test groups and were attached to the screws, according to Figure 1B.

All temperature sensors were read before (t_0) and after (t_1) the TUS application. The procedures of each group were repeated three times on each sample, while keeping the same order so that the temperature of the first TUS application would normalize and not interfere with the temperatures of the second and third replications. The average of the three replications for all temperature sensors and the difference between them (t_1-t_0) were calculated.

For the statistical analysis, the temperature differences (t_1-t_0) of sensors from each sample were used, and the one-way analysis of variance (ANOVA) was applied, followed by Duncan's multiple range test. All results are shown as the mean±standard error of the mean (SEM), with a 5% significance level.

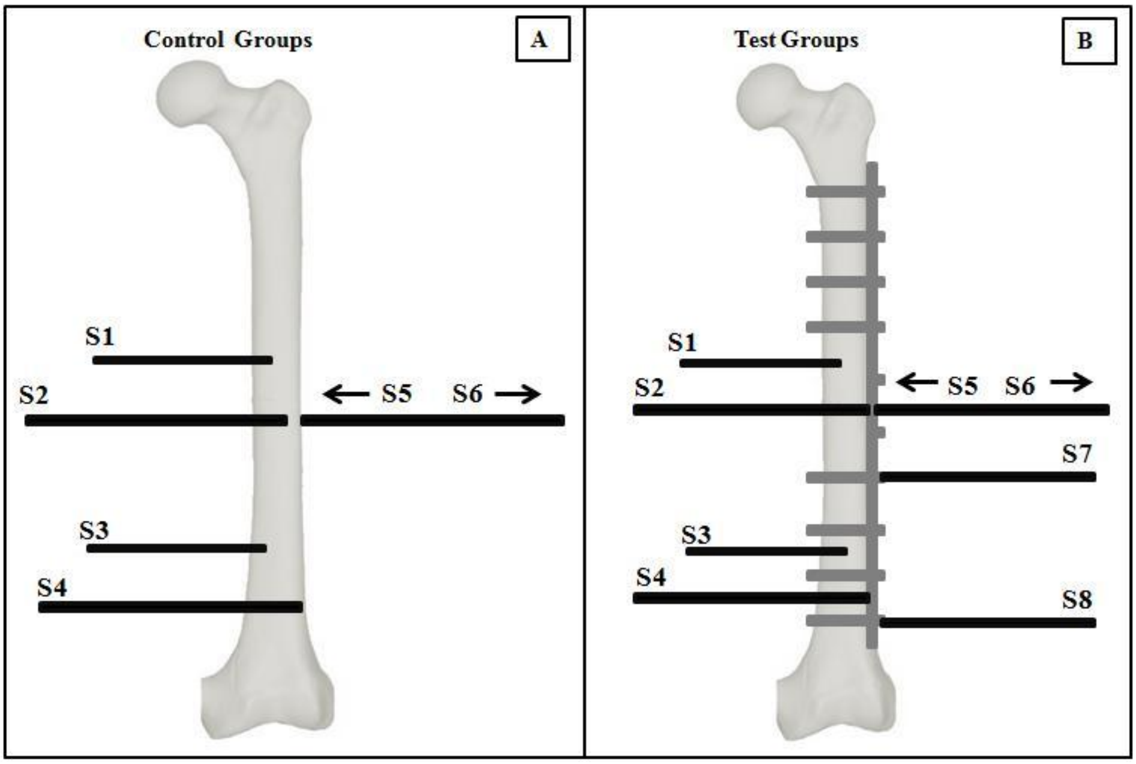

Figure 1. Schematic representation of the femur and the placement of the temperature sensors (▬) in the control groups - CGI, CGII, CGIII and CGIV- (A) and test groups -TGI, TGII, TGIII and TGIV- (B). S1: medial side, medullary cavity; S2: medial side, inner lateral cortical bone (control groups), metal plate (test groups); S3: medial side, distal medullary cavity; S4: medial side, distal inner lateral cortical bone (control groups), distal metal plate (test groups); S5: lateral side, cortical bone (control groups), metal plate (test groups); S6: lateral side, intramuscular; S7: lateral side, proximal screw; S8: lateral side, distal screw.

RESULTS AND DISCUSSION

The use of the hind limbs of dog cadavers as an experimental model was efficient and allowed drilling the cortical bone at different sites to place the temperature sensors. It also provided preliminary data that indicated the need to perform the same study "*in vivo*". In humans, Cambier *et al*. (2001) used cadavers for purposes similar to the ones of the present study. The process of freezing and reheating the samples and the absence of blood flow, which assists in dissipating the heat generated by ultrasound according to Baker *et al*. (2001), can be noted as limitations of the present study. The average temperature in all samples evaluated at the moment t_0 was 19.3°C. These factors most likely influenced the temperature values, and the results could be different if the study was conducted in living animals. However, the proposed objective confirmed the heat produced by the TUS in all groups studied, as shown in Table 1, confirming the findings of Garavello *et al*. (1997), who also observed a temperature increase in the muscle, on the plate and in the cortical bone of rabbits after 5 minutes of application of a 875-kHz TUS with intensities of 1 to 3 W/cm².

The intensity and duration of the TUS application defined in the present study were based on a physiotherapeutic routine for small animals (Steiss *et al*., 2004; Canapp, 2007). The duration was selected according to the size of the treatment area and the ERA (Young, 2003). These dosages correspond to the ones frequently used in human sports physiotherapy (Warden *et al*., 2002). Several studies used parameters that extrapolated the normally used values or omitted important information, thereby limiting the contributions of the obtained results, as well as their comparison with those found in the present study (Herrick, 1953; Skoubo-Kristensen *et al*., 1982; Garavello *et al*., 1997).

Regarding sensors S3, S4 and S8, there were no differences when comparing the groups with different frequencies and intensities because their temperatures remained almost unchanged after the TUS application (Table 1). Even with the absorption of ultrasonic energy in the treatment area (S1, S2 and S5), the low dissipation during heat conduction most likely contributed to this result.

Regarding the S1 sensor, Table 1 shows that groups subjected to the 1-MHz frequency and 2-W/cm² intensity showed the highest heating ($P < 0.05$) when compared to the other groups, which was expected due to the higher penetration (frequency) and strength of the sound waves (intensity) in tissues and the consequent absorption of ultrasonic energy (O'Brien Junior, 2007).

Table 1. Mean of the difference (t_1 - t_0) in temperatures (°C) in the samples treated with the therapeutic ultrasound according to the sensors (S) and experimental groups

Group/Sensor	S1	S2	S3	S4	S5	S6	S7	S8
CGI	0.40	0.66	0.06	0.06	1.66	1.84	-	-
CGII	0.80	1.24	0.06	0.04	3.38	3.84	-	-
CGIII	0.14	0.40	0.06	0.08	1.52	2.02	-	-
CGIV	0.30	0.78	0.18	0.04	2.82	3.64	-	-
TGI	0.40	0.34	0.04	0.04	1.48	1.68	0.30	0.14
TGII	0.76	0.98	0.28	0.04	2.78	3.74	0.74	0.06
TGIII	0.14	0.30	0.06	0.08	2.14	2.90	0.22	0.04
TGIV	0.36	0.56	0	0.04	3.50	4.50	0.48	0.02

Control groups: CGI, CGII, CGIII and CGIV; Test groups: TGI, TGII, TGIII and TGIV. S1: medial side, medullary cavity; S2: medial side, inner lateral cortical bone (control groups), metal plate (test groups); S3: medial side, distal medullary cavity; S4: medial side, distal inner lateral cortical bone (control groups), distal metal plate (test groups); S5: lateral side, cortical bone (control groups), metal plate (test groups); S6: lateral side, intramuscular; S7: lateral side, proximal screw; S8: lateral side, distal screw.

Furthermore, the S2 sensor detected greater heating in the control groups when compared to their respective test groups, indicating a possible interference of the metal plate with the sound energy transmission (absorption and reflection). In the present study, the cortical bone, although it is highly reflective as mentioned by Lehmann *et al*. (1958), absorbed more sound energy than the metal plate. The same explanations mentioned above can be used in the correlation of the S2 and S5 sensors, which showed significant differences ($P<0.05$) in all experimental groups (Figure 2A). On average, the S5 sensor detected 23.6% and 41.8% more heating than the S2 sensor in the 1-MHz control groups (CGI and CGII) and test groups (TGI and TGII), respectively, and the S5 sensor detected 46% and 70% more heating than the S2 sensor in the 3-MHz control groups (CGIII and CGIV) and test groups (TGIII and TGIV), respectively (Table 1).

When analyzing the temperatures measured by sensors S5, S6 and S7 separately (Table 1), the groups subjected to a 2-W/cm² intensity had higher temperatures ($P<0.05$) regardless of the frequency tested. This result can be explained by the intensity-dependent biophysical effects of the TUS: a higher intensity resulted in more energy reaching the treated tissues, as reported by Watson (2008). Additionally, all temperatures detected by sensor S6 (intramuscular) were higher than those detected by sensor S5 ($P<0.05$) (Figure 2B), confirming the results of Garavello *et al*. (1997). However, Herrick (1953) stated that the temperature produced in the cortical bone is significantly higher than the temperature of the surrounding tissues, such as the muscle. Watson (2008) clarified that the absorption capacity of the sound wave is proportional to the protein content, and theoretically, the bone and cartilage tissues would benefit from that effect. However, the author notes that problems associated with the reflection of sound waves in these structures cause significant energy loss, yielding more effective heating in tissues that have higher collagen content. Another possible explanation of the results could be an acoustic interface represented by the bone-soft tissue (control group) and the metal plate-soft tissue (test group) because, according to Young (2003), there is a considerable percentage of ultrasound reflection when it reaches these structures. Lehmann *et al*. (1958) had already reported the phenomenon of reflection, highlighting the considerable increase in the ultrasonic intensity in the presence of metal implants. In this study, the reflected waves possibly reached the muscle, where a portion was absorbed, contributing to the temperature increase. Lehmann *et al*. (1959) performed studies in pigs using different metal implants and concluded that the temperature measured in the muscle was also caused by ultrasound reflections of the metal surface.

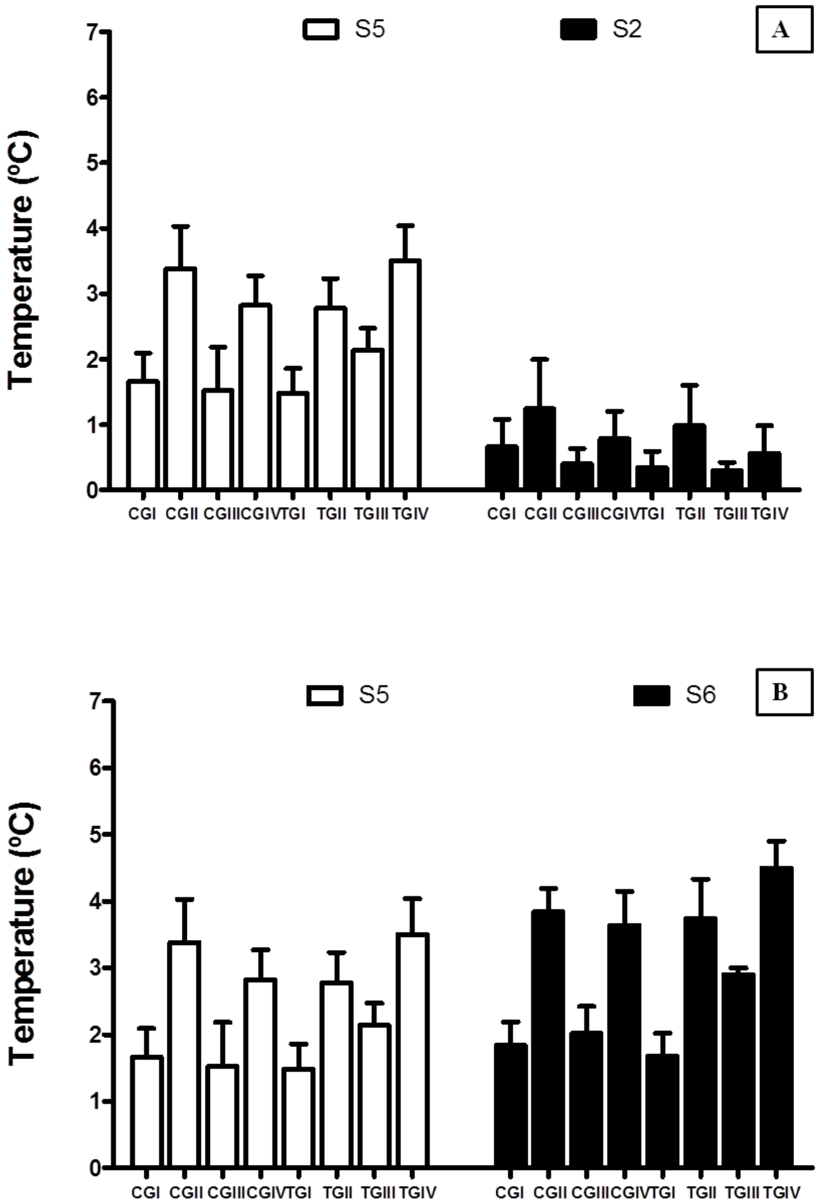

Figure 2. Graphic demonstration of the mean of temperatures in the samples treated with the therapeutic ultrasound according to the sensors and experimental groups. Note in A, the higher temperatures from sensor S5 and, in B, from sensor S6 (intramuscular). There were differences ($P < 0.05$) between sensors S5 and S2 (A) and S5 and S6 (B) in each analyzed group.

When comparing the S6 sensor between the groups, in the 3-MHz frequency, the test groups (TGIII and TGIV) had higher temperatures ($P<0.05$) than their respective control groups (CGIII and CGIV), and the highest temperature was recorded in TGIV, which was treated with a 2-W/cm^2 intensity (Table 1). Nevertheless, the temperatures were below 5°C in all groups (Table 1); such temperatures, according to Draper *et al*. (1995), promote the desired therapeutic effect without causing tissue damage. These same authors observed that, when using the 3-MHz frequency, the muscle heating was three times higher than when using the 1-MHz frequency. However, Cambier *et al*. (2001) stated that a lower frequency corresponded to greater muscle heating in cadavers, which was not confirmed in the present study. Gersten (1958) also conducted studies to verify the intramuscular temperature in dogs subjected to the placement of a metal plate and found higher temperatures in the presence of the implant; they related this result to high temperatures close to the muscle-metal plate interface, which were not observed in the present study (Table 1) because the temperatures in CGI and CGII were higher than in their respective test groups (TGI and TGII).

When comparing the temperatures of sensors S1 and S3 (proximal and distal medullary cavity), S2 and S4 (proximal and distal cortical bone or metal plate) and S7 and S8 (proximal and distal screw) (Figure 1A and 1B), the sensors near the TUS application site (S1, S2 and S7) exhibited higher temperatures in all groups (Table 1). One of the hypotheses that might explain this result would be that the S1, S2 and S7 sensors are near or inside the TUS treatment area; additionally, low heat loss during heat dissipation may have contributed due to the distance between sensors.

The findings of the present study showed that the temperatures after TUS application (t_1) were higher, regardless of the study group. Although the absorption rate increased as the frequency increased, making less energy available for penetration into the tissue (O'Brien Junior, 2007), the temperature recorded by the S6 sensor (intramuscular) reached the highest value (Table 1) in the group subjected to the 3-MHz frequency in the presence of the metal plate (TGIV). Therefore, the 3-MHz TUS application in the continuous mode at sites up to 3-cm thick requires caution in the presence of a metal plate. Further *in vivo* studies with a metal plate placed in the femur of dogs are recommended to assess the tissue behavior when using the TUS at a 3-MHz frequency because there was considerable heating in the muscle tissue near the metal implant.

CONCLUSION

In conclusion, the TUS in continuous mode with 1- and 3-MHz frequencies and 1- and 2-W/cm^2 intensities for two minutes promotes heating of a metal plate and its adjacent structures after fixation to the femur of canine cadavers. Further studies on live animals are required to assess the effects of the heat produced by the TUS on bone healing and the behavior of the adjacent soft tissues in the presence of a metal plate.

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