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Original Article

Effect of Temperature on Ultrasonic Properties of the Calcaneus In Situ

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Abstract. To assess the dependence of calcaneal quantitative ultrasound (QUS) on foot temperature, a series of acoustic measurements were made in five cadaver feet in situ (all soft tissues retained) over a temperature range of 25 °C to 40 °C in steps of 5 °C. An implanted probe was used to measured temperature directly in the calcaneus itself. Ultrasound velocity decreased linearly with increasing temperature, with a mean thermal coefficient of −2.2 m/s/°C. In contrast, broadband ultrasonic attenuation (BUA) increased with temperature with a mean thermal coefficient of +0.75 dB/MHz/°C. We argue that the temperature trends in velocity are likely to be due to the influence of fat, present in the bone marrow and in the soft tissues, which has a negative thermal coefficient for acoustic velocity. The attenuation trends may arise, in part, from greater scattering losses inside the cancellous bone due to an increased acoustic impedance mismatch between trabeculae and fatty marrow at higher temperatures. These considerations suggest that the greatest temperature effects may be expected in patients with a high proportion of fat within the measured volume and/or low calcaneal bone density. Given the magnitude of the thermal coefficients observed, the clinical impact of temperature-related QUS errors is likely to be modest for diagnostic purposes but of greater significance in follow-up studies.

Keywords: Calcaneus; Quantitative ultrasound (QUS); Temperature effects

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Introduction

Quantitative ultrasound (QUS) of the calcaneus is increasingly being used to assess bone status and the risk of fracture, particularly in the context of osteoporosis [1]. Measurements are made in transmission transversely across the foot such that ultrasound propagates through overlying soft tissues as well as through the calcaneus itself. The amount, composition and geometry of the overlying soft tissues, as well as the properties of the marrow within the cancellous bone, may all influence the measurement [2]. The acoustic properties of biological tissues are generally temperature-dependent [3], and therefore it is likely that QUS measurements will be affected by temperature. In subjects whose foot temperature differs from normal, whether due to pathologic or environmental factors, clinical QUS measurements may consequently be subject to errors.

Velocity in fat and fatty tissues decreases with increasing temperature, whereas water, blood and muscle all show the opposite trend [3]. Attenuation decreases with increasing temperature in fat, but generally increases with increasing temperature in more structurally complex tissues such as the liver and kidney [3]. In cortical bone, velocity decreases with increasing temperature at approximately −6 m/s/°C [4], while attenuation shows modest increases [5]. In excised cancellous bone specimens with marrow retained velocity decreases with increasing temperature, but attenuation is reportedly unchanged [6]. In contrast, attenuation in *water-filled* cancellous bone specimens reportedly decreases with increasing temperature [7].

In vivo measurements of the human calcaneus suggest a negative thermal coefficient for velocity and a positive thermal coefficient for attenuation [8–10]. However, these findings are ambiguous since calcaneal temperature was inferred from water bath or skin temperatures rather than measured directly. The only in situ data available are those obtained for a single cadaver foot, which yielded results broadly in line with the reported in vivo trends, but, again, temperatures were not measured directly in the calcaneus [10]. Furthermore, it is notable that, with a single exception [11], there have been no attempts to develop physical explanations for these temperature trends. Hence, in this study we aimed to quantify the temperature dependence of the ultrasonic properties in five intact cadaver feet using direct temperature measurements, with the aim of improving our understanding of the magnitude of these effects, their potential clinical significance and their underlying causes.

Materials and Methods

Measurements were made on five cadaver feet (4 female, 1 male; ages 81–91 years) cut at mid-tibia. The specimens had all soft tissues intact and were stored frozen in sealed plastic bags. Prior to measurements, each foot was removed from the freezer and a temperature probe was implanted into the anterior region of the calcaneus near the tarsal sinus through a 10 mm diameter drilled hole, which was then tightly packed with gauze. Fluoroscopy was used to confirm the location of the probe (Fig. 1). The temperature probe was part of a standard aquarium thermometer having a digital temperature display with a precision of 0.1 °C. Accuracy was better than 0.2 °C over the temperature range used in the study, as assessed against a calibrated laboratory electronic thermometer. The feet were thawed

Fig. 1. Fluoroscopic lateral image of a cadaver foot showing the temperature probe implanted in the calcaneus near the tarsal sinus.

overnight (14–16 h) at room temperature prior to the measurements described below.

Ultrasonic measurements were made with an in-house pulse transmission system using a pair of 12.5 mm diameter 1 MHz broadband unfocused transducers mounted coaxially 130 mm apart. Measurements were made in a water bath equipped with an immersion heater/circulator that controlled temperature with a precision of 0.1 °C. Velocity was determined from transit time measurements using the first zero-crossing point of the waveform [12]. The foot width, measured with calipers at the QUS measurement site (see below), was used in deriving velocity. Broadband ultrasonic attenuation (BUA), calculated as the linear slope of attenuation versus frequency from 300 to 750 kHz, was determined from the power spectra of pulses transmitted through water and through the foot.

Throughout the experiment the foot was strapped onto a rigid acrylic frame which locked into the base of the water tank, ensuring reproducible orientation of the frame and foot relative to the transducers. The anatomic site measured was located 34 mm from the bottom of the foot and 30 mm forward from the back of the heel, and was designed to match that used by the Sahara clinical sonometer (Hologic, Bedford, MA). Heel thickness was measured at this location using digital calipers (five repeat measurements) at the beginning of the experiment and again after all the ultrasonic measurements were completed.

Four temperatures were studied (25, 30, 35 and 40 °C). For each temperature studied, the foot, strapped on the acrylic frame throughout, was placed in a plastic bag in the water bath, and the water bath was warmed rapidly to the target temperature. We then waited until approximate thermal equilibrium was reached, defined as a calcaneal temperature within 1 °C of the water bath temperature. Thermal equilibrium was so defined in order to limit the time spent waiting for the foot to warm to the required temperature, given that excessively long waiting periods would have increased the risk of unwanted physical changes in the specimens. The foot was then removed from the bag and five repeat ultrasonic measurements were made, with repositioning between measurements. The foot was then removed from the water, dried with a towel, and placed back in the plastic bag for warming to the next temperature. By keeping the foot in the bag during the warming periods, the time spent in direct contact with water was minimized, with the intention of reducing the potential for edema and changes in skin acoustic properties.

To minimize the possible effects of air bubbles, the water tank was allowed to stand for at least 12 h prior to measurements to allow air to come out of solution. In addition, both the transducers and the skin surface were wiped with soaked gauze prior to each QUS measurement in order to remove any remaining air bubbles.

For each specimen, the mean of the five repeated acoustic measurements at each temperature was calculated, and then the thermal coefficients were estimated as the slope of the linear regression of the acoustic parameter (velocity or BUA) against temperature. Differences in heel thickness before and after the experiment were assessed using a paired *t*-test.

The short-term reproducibility with repositioning in QUS measurements using the methodology outlined above was 0.1% for velocity and 1.4% for BUA (RMS CV).

In order to provide a clinically relevant measure of the degree of calcaneal osteopenia in these feet from elderly subjects, bone mineral density (BMD) was measuring using dual-energy X-ray absorptiometry (QDR-2000+, Hologic Inc., Bedford, MA). The foot was scanned in air in the lateral projection using the forearm scanning mode. BMD was measured within a circular region of interest centered on the QUS measurement site.

Results

For each specimen the measurement sequence took approximately 9 h to complete. After each 5 °C increment in water bath temperature it typically took 2 h for the calcaneal temperature to rise to approximate thermal equilibrium (i.e. to within 1 °C of the water temperature). None of the specimens exhibited a significant change in heel thickness during the experiments.

In all five specimens velocity decreased with increasing temperature ($p<0.01$) (Table 1, Fig. 2). Thermal coefficients ranged from –1.8 to −2.8 m/s/°C. The mean (standard deviation) for the five specimens was −2.2 (0.4) m/s/°C. The correlation coefficients between velocity and temperature ranged from r = −>0.98 to −1.00. In contrast, positive thermal coefficients were observed for BUA, ranging from +0.21 to +1.74 dB/MHz/°C, with a mean value of +0.75 (0.59) dB/MHz/°C for the five specimens studied (Table 1, Fig. 2). Correlation coefficients between BUA and temperature ranged from r = +0.86 to +1.00.

The calcaneal BMD ranged from 0.28 to 0.60 g/cm^2 (Table 1), with a mean of 0.45 g/cm^2.

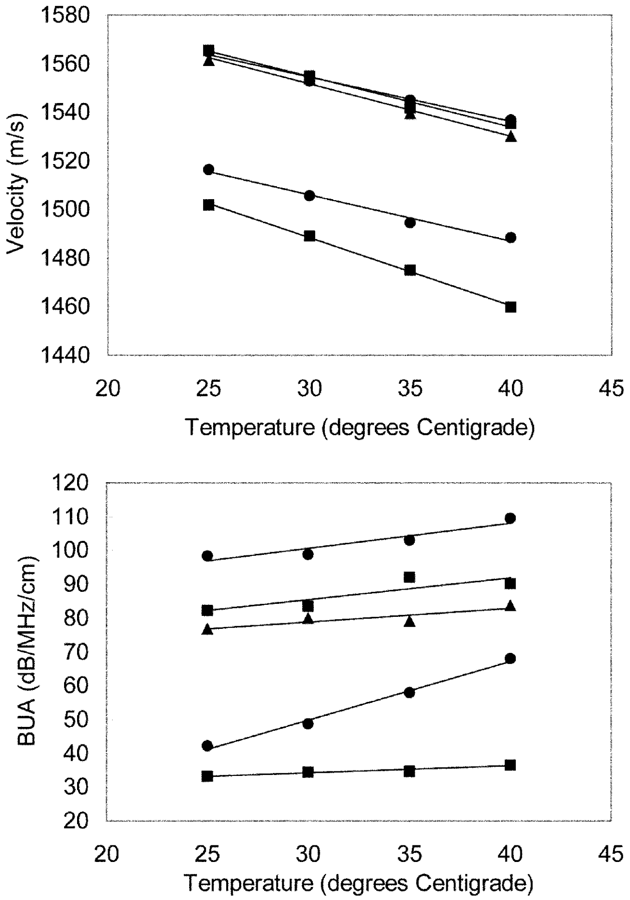

Fig. 2. Dependence of ultrasonic velocity and BUA on temperature in five cadaver feet. Each data point shown is the mean of five repeat measurements made in each foot at each temperature. Linear fits to the data are shown as continuous lines, the slopes of which represent the thermal coefficients. See also Table 1.

Discussion

This study has presented the first in situ cadaver data on relationships between directly measured calcaneal temperature and calcaneal ultrasonic properties. Average thermal coefficients of −2.2 m/s/°C were observed for

Table 1. Acoustic properties and their thermal coefficients in five cadaver feet

Specimen	BMD (g/cm^2)	Velocity[a] (m/s)	Velocity ϕ[b] (m/s °C)	BUA[a] (dB/MHz)	BUA ϕ[b] (dB/MHz/°C)
A. Female 87 years	0.559	1545.1 ± 2.0	−1.8 (0.1)	103.0 ± 1.7	+0.75 (0.19)
B. Male 81 years	0.595	1541.8 ± 2.0	−2.1 (0.2)	92.1 ± 0.8	+0.65 (0.27)
C. Female 80 years	0.477	1539.6 ± 1.5	−2.2 (0.2)	79.2 ± 1.0	+0.40 (0.14)
D. Female 86 years	0.355	1494.5 ± 1.0	−1.9 (0.2)	58.0 ± 0.7	+1.74 (0.11)
E. Female 91 years	0.283	1475.0 ± 1.1	−2.8 (0.1)	34.7 ± 0.2	+0.21 (0.04)

[a] Quoted values are mean ± standard deviation for five repeat measurements (with repositioning) at 35 °C.
[b] ϕ is the thermal coefficient determined from the least squares linear fit to the data (averaged at each temperature) from 25 to 40 °C (see Fig. 1). The standard error is shown in parentheses.

velocity, and +0.75 dB/MHz/°C for BUA. One weakness of the study is that only five specimens were measured. Reasons for the small number of specimens included the fact that the experiments were time-consuming and demanding (each experiment taking 10–12 h in total to perform), and the limited availability of cadaver material. However, given that all five specimens showed the same trends with regard to the temperature-dependency of acoustic properties, it is reasonable to assume that these trends are representative. In addition, the calcaneal BMD values of the five feet spanned the full range seen clinically in elderly subjects [13], suggesting that, although small in number, these specimens were representative of the elderly population, at least in terms of BMD. Barkmann and Glüer [8] reported thermal coefficients for calcaneal acoustic properties in vivo (−3.6 ± 0.5 m/s/°C for velocity, +0.27 ± 0.09 dB/MHz/°C for BUA) that are of the same order as our in situ data, despite using skin surface temperatures. It therefore appears that the in vivo and in situ situations are to some extent comparable.

The negative temperature dependency that we observed for acoustic velocity is likely due to the influence of fat within the measured volume, both in the overlying tissues and in the bone marrow. This is because, as noted earlier, fat has a negative thermal coefficient for velocity between 20 and 40 °C, whereas thermal changes in blood and muscle are in the opposite direction [3]. If this hypothesis is correct, then QUS temperature changes can be expected to be greater in patients who have a greater proportion of fat in the overlying tissue or in the calcaneus. The latter could arise either from increased marrow adiposity or through a greater relative marrow volume (i.e. low bone density).

A positive thermal coefficient for BUA was observed in all five specimens studied, providing confirmation of trends earlier reported for a single cadaver foot [10]. To date, it appears that no explanation for this behavior has been proposed. We suggest that changes in attenuation due to scattering inside cancellous bone could explain the observed behavior. Scattering depends in part on the difference in acoustic impedance between the trabeculae and the marrow fluid. If the sound speed in marrow decreases with temperature (as it will if the marrow is mostly fat), then scattering losses will increase with temperature. Using a simple scattering model that we have described elsewhere [14], this effect can be evaluated quantitatively. Assuming (a) a sound speed and thermal coefficient for marrow of 1470 m/s and −5 m/s/°C respectively, (b) corresponding values for trabeculae of 3300 m/s and 0 m/s/°C, and (c) cancellous bone porosity of 80% and a scatterer size of 0.3 mm, then the predicted thermal coefficient for BUA due to changes in scattering is approximately +0.3 dB/MHz/°C. This is of the same order as the coefficients measured experimentally, thus confirming the plausibility of the hypothesis. Furthermore, the predictions are not substantially altered if a negative thermal coefficient (e.g. −5 m/s/°C) is assumed for trabeculae. One implication of this hypothesis is that subjects with a greater proportion of fat in the bone marrow could be expected to show greater temperature-related changes in BUA. We note that recent work tends to confirm the importance of the role of marrow in determining the acoustic properties of trabecular bone, in that ultrasound velocity was significantly lower in marrow-filled bone compared with water-filled bone, but attenuation and backscatter were increased [15].

Given the short time-scale involved (of the order of minutes), it would appear that temperature-related changes in calcaneal bone acoustic properties cannot explain the reported rapid variation in velocity and BUA (measured in vivo) immediately following immersion of the foot in the water bath of a QUS device [9,16]. These rapid effects indicate changes occurring in the superficial tissue rather than in the bone. These could be changes in acoustic properties due to direct temperature effects, or could be mediated through mechanisms such as the passive interaction of water with the skin or various active physiologic responses. The point to be made is that QUS is a measurement made through the whole foot, and can be expected to reflect changes in the overlying soft tissues as well as in the calcaneal bone itself.

The variation in QUS measurements with foot temperature represents a potential source of error in clinical measurements. Foot temperature may differ between individuals, and in the same individual measured at different times, due to pathologic factors (e.g., diabetes mellitus, peripheral vascular disease, fever) or environmental and behavioral factors (e.g., ambient temperature, foot attire, exercise). Data on inter- and intra-subject variations in foot temperature are needed to assess the clinical implications of our findings, but very little information is available in the literature. Infrared noncontact temperature measurements of the foot surface in healthy subjects yield temperatures ranging from 22 to 34 °C, with a mean of 28 °C (R. Barkmann, personal communication). However, these are surface temperatures and the temperature of the calcaneus itself is likely to be higher and more constant. Nevertheless, if we take a 5 °C calcaneal temperature difference as plausible, our data indicate that corresponding changes in acoustic properties of the order of 10 m/s and 5 dB/MHz for velocity and BUA respectively. Expressing these values as *t*-scores (dividing them by the population standard deviation for heel QUS in young normals, typically 25 m/s for velocity and 15 dB/MHz for BUA [17]), yields values of 0.3–0.4. This suggests that diagnostic misclassification due to temperature effects is likely to be small. However, where QUS is used in a monitoring role, temperature effects may be much more important given the enhanced need for precision relative to the small changes expected [18].

Temperature effects on QUS are not restricted to human feet – temperature effects have been observed in QUS quality assurance phantoms [19], and, presumably, may also occur in QUS measurements of excised trabecular bone samples. Interestingly, QUS phantoms appear to behave similarly to cadaver feet, displaying a negative thermal coefficient for velocity and a positive coefficient for BUA [19].

One approach to minimizing QUS temperature-related errors would be to bring the foot to a known uniform temperature prior to measurement. However, given the length of time needed to bring a cadaver foot into thermal equilibrium (at least 2 h based on our findings), and the potential complicating role of physiologic thermoregulation, this may be impossible to achieve in practice. Immersion of the foot in a constant-temperature water bath for a specified time prior to measurement would at least represent a partial step in this direction. This would obviously be easier to implement in water-coupled, rather than gel-coupled QUS systems. An alternative solution could be to use ultrasonic indices that combine velocity and BUA in such a way that temperature effects are, to some extent, cancelled out [9]. On the other hand, this seems premature given that we do not fully understand the causes of temperature-related changes in foot acoustic properties. There are strong arguments for seeking these causes rather than simply attempting to correct for them empirically. Whilst temperature changes in ultrasonic properties may complicate clinical measurements of the calcaneus, they may in fact provide valuable insights into the underlying acoustic interactions.

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