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Influence of Fat on Ultrasound Measurements of the Os Calcis

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Abstract. Measurements of the speed-of-sound (SOS) and of the broadband ultrasound attenuation (BUA) on the os calcis were recently proposed to assess osteoporotic fragility. Velocity and attenuation were measured through the heel which can be divided in three phases including hydroxyapatite, soft tissue, and fat. The aim of this study was to evaluate the influence of fat composition and heel width on SOS and BUA. This influence was determined from both *in vitro* investigations examining fat samples, phantoms, and cadaver heels, and *in vivo* ones observing adult volunteers as well as a wide sample section of healthy elderly women. Ultrasound velocities on various fat samples were significantly lower than those on distilled water (−65 m/second to −123 m/second). The excision of the surrounding soft tissue from cadaver heels made SOS steadily increase whereas the insertion of a 10 mm piece of lard in the lateral face of cadavers' and volunteers' heels os calcis lowered SOS about 30 m/second. Furthermore, a difference of SOS was estimated at 15 m/second for a 12.5% variation of the marrow fat weight. Among 334 elderly and healthy women aged 75 and over, a significant negative correlation was found between SOS and heel width ($r = -0.27$; $P < 0.0001$). On the other hand, fat composition had no significant effect on BUA measurement, and no significant relationship was found between BUA and heel width. This study demonstrates that an increase of heel width and fat thickness provides an underestimation of os calcis SOS, but has no significant effect on BUA.

Key words: Fat — Ultrasound attenuation — Speed-of-sound — Os calcis.

Detecting women who are at increased risk of osteoporosis is as a major medical concern. The age-related bone mass loss, revealed by dual-energy X-ray absorptiometry (DXA) [1], is an important risk factor of spinal or hip fracture [2, 3]. Combined information about elasticity, structure, and density may provide a more sensitive indicator of fracture risk than techniques reflecting density alone. Langton et al. [4] suggested that measurement of broadband ultrasound attenuation can give a direct interpretation of the bone structure as well as its density. On the other hand, the relationship between ultrasonic velocity and cancellous bone strength has been demonstrated by Turner and Eich [5], and speed-of-sound (SOS) is influenced by the bone elasticity and density [6].

Applications of sonic methods for the clinical study of bone are now available. Most of the ultrasound densitometers recently developed measure the os calcis, which is an accessible, relatively large, cancellous bone containing a high percentage of trabecular bone (90%). But, the ultrasound densitometers provide broadband ultrasound attenuation (BUA) and/or SOS through the heel which can be divided into three phases: hydroxyapatite, soft tissue, and fat (marrow and subcutaneous). These phases are not taken into account in the ultrasound measurement. However, soundwaves speed is higher through relatively dense objects than through less dense, and consequently more flexible ones. So it seems necessary to interpret BUA and SOS according to the heel tissue composition. McCloskey et al. [7] found a high concordance in BUA between measurements *in vivo* and measurements of fully dissected calcaneus, suggesting that the presence of surrounding soft tissue does not disturb the results. Bradenburger et al. [8] reported that overlying soft tissues and small variations of the heel thickness had an adverse effect on SOS but not on BUA. However, these authors have not considered the influence of the soft tissue composition.

The purpose of this study was to accurately evaluate the influence of fat and heel width on SOS and BUA of the os calcis. This influence will be determined from both *in vitro* studies, using fat samples, phantoms, and cadavers heels, and *in vivo* studies on adult volunteers, and a wide sample section of healthy elderly women.

Materials and Methods

In Vitro Studies

Reference Materials. The SOS and BUA of distilled water, glycerol trioleate ($C_{57}H_{104}O_6$), castor oil, and pork lard were determined. Densities (d) at 35°C were determined using Archimedes' principle and are reported in Table 1. Glycerol trioleate is classicaly used to simulate human fat ($d = 0.91$ g/cm^3 at 35°C) [9] in experimental radiation physics such as DXA [10]. Castor oil is appropriate to simulate bone marrow ($d = 0.95$ g/cm^3 at 35°C). Glycerol trioleate and castor oil are liquid at room temperature and at 35°C. Pork lard is solid at room temperature and at 35°C. Each fat sample was 4 cm width. To establish SOS and BUA as a function of temperature, reference materials were measured in the range 29°–37°C. In order to evaluate influence of fat with accuracy, SOS and BUA were calcu-

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Table 1. Density, SOS (X ± SD), and BUA (X ± SD) for distilled water and fat samples measured 20 times at 35°C

Sample	Density	SOS (m/second)	BUA (dB/Mhz)
Water	1.00	1525 ± 6.0	56.6 ± 0.4
Castor oil	0.95	1460 ± 1.9	57.4 ± 0.7
Pork lard	0.93	1422 ± 3.3	55.4 ± 0.7
Trioleate	0.91	1402 ± 2.9	57.7 ± 0.6

lated from 20 measures at 35°C, which is the standard temperature of os calcis measurements.

Phantom. The Phantom was composed of 25.5 mm of polyurethane and pieces of lard of different thickness (0, 10, and 20 mm). The polyurethane part stood for the quality assurance standard of the Lunar Achilles® ultrasonic densitometer. SOS and BUA of the standard were in the range of human heel normality. SOS and BUA were also calculated at 35°C from 20 measures.

Cadaver Heels. The influence of surrounding fat tissue was examined in the os calcis by measuring four fresh cadaver heels (within 6 hours). First, both BUA and SOS determinations were performed in the cadaver heels before and after excision of the surrounding soft tissue; then measurements were repeated after insertion of a 10-mm piece of lard in the lateral face of the os calcis. After the ultrasonic measurements, a cylinder drill was used to obtain a bone cylinder from each fresh os calcis, then the total weight of each cylinder, standing for the bone in the measuring path, was measured. After the ethanol bath (100% vol), fat was removed from each cylinder by toluen dissolving. The heel bone cylinders were dried at room temperature for 12 hours and at 37°C for 2 hours, then the dried heel bone cylinders were weighed. The weight of marrow fat expressed in percentage of total weight was calculated by difference.

In Vivo Studies

Volunteers. Thirty healthy volunteers (6 males and 24 females) aged 37 ± 7 years, range 29–47, working in the Nuclear Medicine Department, gave their informed consent. Both SOS and BUA of the right heel were measured before and after insertion of a 10-mm piece of lard.

Elderly Women. Three hundred thirty-four healthy female volunteers aged 80 ± 4 years, range 75–97, were recruited through multicenter prospective epidemiological studies (named EPIDOS) on the risk factors for hip fracture among elderly women aged 75 and more. The volunteers were randomly selected from the voting lists. Excluded were those women who had a history of hip fracture, hip prothesis, Paget's and malignant bone disease, and renal failure. No therapy was considered as an exclusion criterium. In these 334 women the heel width was measured manually 2 cm below the external malleolus and 3.7 cm from the back end of the heel using a Lange skinfold calipers. Heel width precision expressed as a coefficient of variation was CV = 2.3%.

Ultrasound Measurements

The measurements were performed using the Lunar Achilles® system (Lunar Corporation, Madison, WI, USA) which consists of two unfocused transducers 2.54 cm in diameter mounted coaxially approximately 9.5 cm apart. Acoustic coupling is accomplished by submerging the transducer pair and the heel into a water bath maintained at 35°C, with surfactant to keep the foot wet. The heel is positioned between the transducers, with the ultrasound beam from the transmitter transducer propagating laterally through the center of the os calcis.

To determine attenuation, the frequency spectrum of the transmission signal was calculated with computer software using a Discret Fourier Transform (DFT) algorithm. The frequency spectrum of the signal once having passed through the heel was substracted from that of the reference waveform (no heel in the propagation path) and a linear regression line was fitted to the amplitude against frequency attenuation data. BUA expressed in decibel per megahertz (dB/MHz) was the slope of this line.

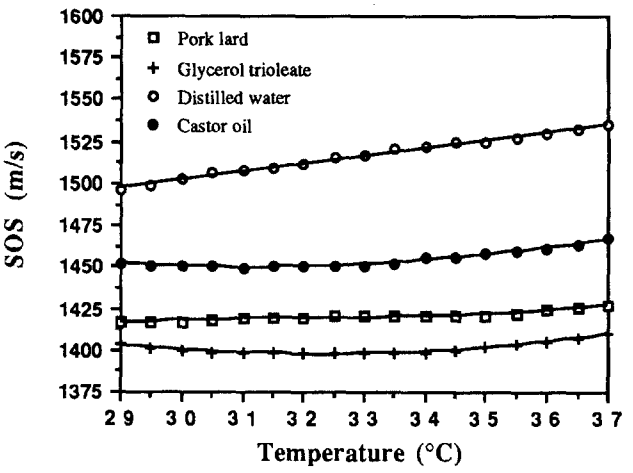

Fig. 1. Variation of speed-of-sound with temperature (29°–37°C) in distilled water and fat samples.

The SOS of the pulse through the heel was calculated by using a "corrected substitution method" reported by Zagzebski et al. [11]. The transit times of the pulse between the transducers was recorded with and without the heel in the propagation path (Δt). The SOS, expressed in m/second, was then calculated as follows:

$$SOS = \frac{C_w}{1 - (C_w \Delta t / d_m)} \quad (1)$$

where C_w is the corrected speed of sound in distilled water at temperature measurement (T), and d_m is the heel thickness defined as a constant, and equal to 4 cm by the manufacturer. In fact, the SOS formula is completed by some adjustments done by Lunar and based on the Quality Assurance Files and on the following equation published by Greenspan and Tschigg [12]:

$$SOS_w = 1402.74 + 5.033T - 0.58T^2 + 0.000332T^3 \quad (2)$$

where T is the water temperature in degrees celsius at 1 atm measured by a temperature captor in the water bath.

In vitro, precision was CV = 0.84% for BUA and CV = 0.12% for SOS [13]. *In vivo* short-term precision for the 30 healthy volunteers was CV = 1.6% for BUA, and CV = 0.2% for SOS.

Statistical Analysis

Statistical analysis was carried out on a microVAX computer with the statistical package SAS version V6.06. The significance of group differences was determined by Wilcoxon signed rank test, and the significance of the correlations was tested by linear or polynomial regression.

Results

SOS and BUA in Reference Materials

Velocity values as a function of temperature in reference materials at 1 atm are shown Figure 1. For distilled water (w) over the range 29°–37°C, variation of SOS with temperature

Table 2. SOS (X ± SD) and BUA (X ± SD) for phantom with different lard thicknesses measured 20 times at 35°C

Lard thickness (mm)	SOS (m/second)	BUA (dB/Mhz)
0	1592 ± 5.0	86.5 ± 0.7
10	1570 ± 4.2	86.1 ± 1.2
20	1543 ± 3.6	85.8 ± 0.7

(T) were satisfactorily estimated by using a linear fit, with the following equation:

$$SOS_w = 1363.32 + 4.66T \ (r = 1, P < 0.0001).$$

SOS variations of castor oil (co), pork lard (pl), and glycerol trioleate (gt) with temperature were best described by cubic curves:

$$SOS_{co} = 2031.75 - 41.25T + 0.868T^2 - 0.0042T^3 \quad (r = 0.99, P < 0.0001)$$
$$SOS_{pl} = 1159.23 + 26.72T - 0.929T^2 + 0.0108T^3 \quad (r = 0.96, P < 0.0001)$$
$$SOS_{gt} = 1282.02 + 25.75T - 1.262T^2 + 0.0173T^3 \quad (r = 1, P < 0.0001).$$

The SOS values of the different reference materials at 35°C (20 measures) are presented in Table 1. Linear and polynomial regressions showed no significant relationship between reference materials BUA and temperature ($P > 0.05$). At 35°C, BUA of the different materials was very close to each other (Table 1).

Influence of Fat in Phantom

At 35°C, there was substantial and progressive decrease of phantom SOS when the amount of fat varied between 0 and 20 mm; but this fact did not affect the measured BUA (Table 2).

Influence of Fat in Cadaver Heels

Results from the SOS and BUA measurements as well as the fat content of the os calcis in the measuring path of four cadaver heels are presented in Figure 2a,b. The excision of the surrounding soft tissue made SOS steadily increase (+11 to +28 m/second) in relation to the presence of fat. On the other hand, the insertion of a 10-mm piece of lard made the SOS decrease (−22 to −38 m/second). For the BUA, no specific variations were noticed after excision of soft tissue (−7 to +2 dB/MHz) or after insertion of lard (−3 to +5 dB/MHz).

Influence of Addition of Fat in Volunteers' Heels

The BUA and SOS of all volunteers' heels were measured twice without insertion of lard (measurements 1 and 2). The two SOS mean values were $SOS_1 = 1587 \pm 44$ m/second and $SOS_2 = 1589 \pm 48$ m/second. *In vivo,* short-term precision expressed as a standard deviation (SD) was 3 m/second for SOS. When inserting 10 mm of lard (measurement 3), the SOS values decreased to $SOS_3 = 1553 \pm 42$ m/second. Wilcoxon signed rank test with paired observations demonstrated that the differences (SOS_1–SOS_3) between the measurements with and without insertion of lard were significant ($P < 0.0001$). Moreover, these differences were significantly higher than the differences of the two measurements (SOS_1–SOS_2) without insertion of lard ($P < 0.0001$). This demonstrated that the effect of insertion of a 10-mm piece of lard was independent of the SOS short-term precision.

The two calculated BUA mean values were $BUA_1 = 120 \pm 11$ dB/MHz and $BUA_2 = 118 \pm 12$ dB/MHz, respectively, without insertion of lard, and $BUA_3 = 118 \pm 11$ dB/MHz with insertion of lard. *In vivo,* short-term precision was SD = 1.9 dB/MHz for BUA. These variations (BUA_1–BUA_3) between the measurements with and without insertion of lard were not significant ($P = 0.495$).

Influence of Heel Width

The mean value of heel width measured on 334 elderly women from the EPIDOS study was 4.9 ± 0.5 cm (range 3.8–6.7 cm). Significant negative correlation was found (Fig. 3a) between SOS and heel width ($r = -0.27$; $P < 0.0001$), and no significant relationship (Fig. 3b) was found between BUA and heel width.

Discussion

The analysis of the results on phantom, cadaver, and volunteer heels confirms the important effect of fat thickness on SOS and the nonsignificant effect on BUA. Within the range 29°–37°C, measured water SOS values increase with temperature and are close to the calculated values from equation 2. SOS is slightly less influenced by temperature in fat than in water. The parabolic shape of the curves describing SOS in glycerol trioleate and castor oil as a function of temperature suggests a solid-liquid phase transition at a temperature within the range 29°–37°C. On the other hand, there is no significant temperature influence on BUA in fat and water.

At 35°C, fat SOS values are in the range of the data (1350–1480 m/second) reported by several authors [14–16]. Velocity in glycerol trioleate and castor oil is always inferior to velocity in distilled water, which explains the influence of bone marrow fat thickness on SOS measurements of the os calcis. In this study, the mass value of marrow fat is consistent with the one indicated by Jonson et al. [17] that reaches about 700 mg/cm^3, and a difference of SOS is estimated at 15 m/second for a 12.5% variation of the marrow fat weight. Besides, insertion of a piece of lard in phantom and volunteers' heels causes a reduction of velocity, which explains the acceleration of SOS in the cadaver heels after excision of the surrounding soft tissue. As a consequence, the fat-induced decrease of apparent SOS of the os calcis depends on the thickness of the subcutaneous fat. Therefore, both surrounding fat tissue and bone marrow fat alter the effective heel SOS and are major sources of error. Otherwise, the negative correlation between heel width and SOS in elderly women confirms this fact, considering that total fat thickness is roughly increasing with heel width.

It is important to view fat influence relative to the normal-osteoporotic difference in order to evaluate the magnitude of this alteration. Thus, changes of 10 mm in subcutaneous fat thickness will provide a decrease of 30 m/second, similar to the normal-osteoporotic difference [18]. On the other hand, this value corresponds to the SOS decrease with age during 2 decades reported by Schott et al. [13]. Influence

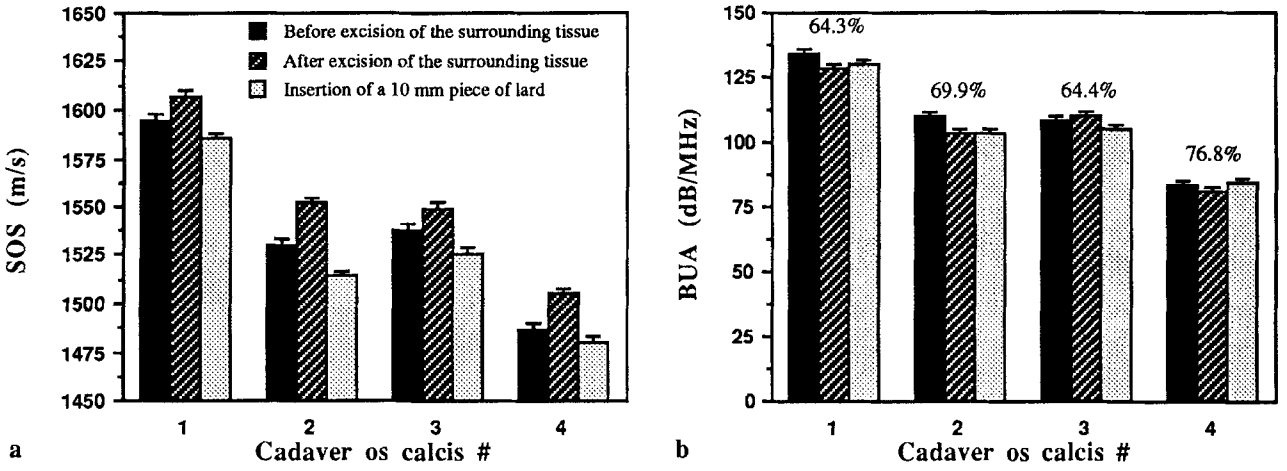

Fig. 2. Influence of soft tissue excision and fat insertion on SOS (a) and BUA (b) in four cadaver heels. For each os calcis, percentage of marrow fat weight is indicated.

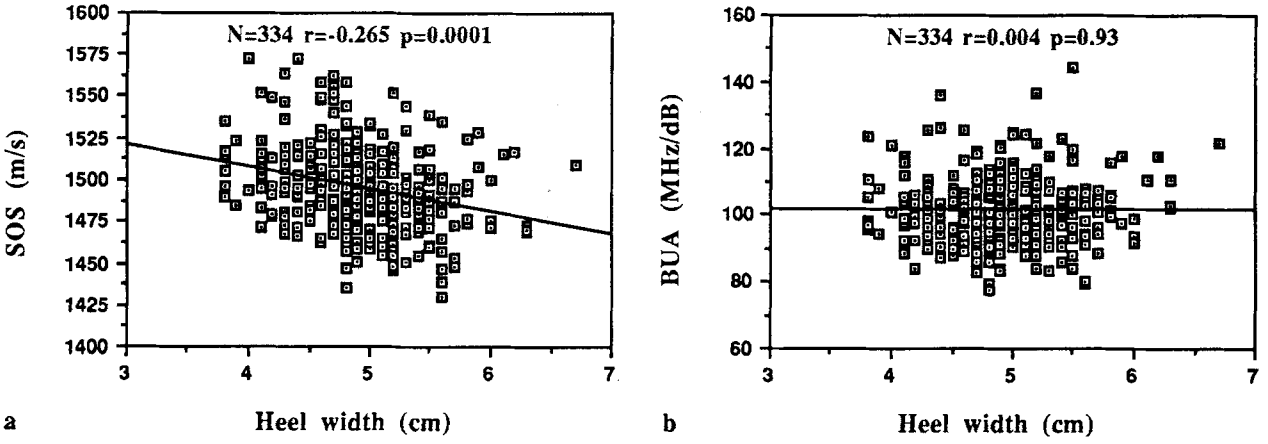

Fig. 3. (a) Significant correlation between SOS and heel width in healthy elderly women. (b) Nonsignificative correlation between BUA and heel width in the same population.

of fat on BUA of the heel is low, and relatively small regarding the normal-osteoporotic BUA difference (17.5 dB/MHz) reported by Agren et al. [19], and the decreasing with age (−4 dB/MHz by decade) [12]. As a consequence, fat composition does not significantly affect ultrasound attenuation of the heel.

To reduce influence of soft tissue and bone thickness, Bradenburger et al. [8] proposed to correct velocity by using the time delays of reflections from the bone surfaces. They concluded that uncorrected velocity has a strong but occult dependence on bone width, but they did not consider the subcutaneous and marrow fat composition. Unfortunately, even with DXA, heel fat composition is difficult to obtain *in vivo*.

In conclusion, neither heel width nor fat composition have significant effect on the BUA measurement. This ultrasound measurement can be used without any correction. Conversely, the increase of the heel width and the fat thickness provides an underestimation of SOS. The first level of correction based on heel width recently proposed by some manufacturers is not sufficient. A second level of correction should be calculated from the fat composition of the heel. In the meantime, failure to correct for the fat composition may result in uncertained interpretation of SOS value.

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References

1. Mazess RB, Barden H, Ettinger M, Schultz E (1988) Bone density of the radius, spine, and proximal femur in osteoporosis. J Bone Miner Res 3:13–18
2. Mazess RB, Collick B, Trempe J, Barden H, Hanson J (1989) Performance evaluation of a dual-energy X-ray bone densitometer. Calcif Tissue Int 44:228–232
3. Cummings SR, Black DM, Nevitt MC (1990) Appendicular bone density and age prediction hip fracture in women. JAMA 263: 665–668
4. Langton CM, Palmer SB, Porter RW (1984) The measurement of broadband ultrasonic attenuation in cancellous bone. Eng Med 13:89–91
5. Turner CH, Eich M (1991) Ultrasonic velocity as a predictor of strength in bovine cancellous bone. Calcif Tissue Int 49:116–119
6. Biot MA (1962) Generalized theory of acoustic propagation in porous dissipative media. J Acoust Soc Am 34:1254–1264
7. McCloskey EV, Murray SA, Charlesworth D, Miller C, Fordham J, Clifford K, Atkins R, Kanis JA (1990) Assessment of

broadband ultrasound attenuation in the os calcis in vitro. Clin Sci 78:221–227
8. Bradenburger G, Waud K, Baran D (1992) Reproducibility of uncorrected velocity of sound does not indicate true precision. J Bone Miner Res (suppl 1):S184
9. Mazess B, Vetter J, Weaver DS (1987) Bone change in oophorectomized monkeys: CT findings. J Comput Assist Tomogr 11:302–305
10. White DR (1978) Tissue substitutes in experimental radiation physics. Med Phys 5:467–478
11. Zagzebski JA, Rossman PJ, Mesina C, Mazess RB, Madsen EL (1991) Ultrasound transmission measurements through the os calcis. Calcif Tissue Int 49:107–111
12. Greespan M, Tschigg CE (1959) Table of speed of sound in water. J Acoust Soc Am 31:75–76
13. Schott AM, Hans D, Sornay-Rendu E, Delmas PD, Meunier PJ (1993) Ultrasound measurements on os calcis: precision and age-related changes in a normal female population. Osteoporosis Int 3:249–254
14. Goss SA, Johnston RL, Dunn F (1978) Comprehensive compilation of empirical ultrasonic properties of mammalian tissues. J Acoust Soc Am 64:423–457
15. Johnston RL, Goss SA, Maynard V, Brady JK, Frizzell LA, O'Brien WD Jr, Dunn F (1979) Elements of tissue characterization, part I: ultrasonic propagation properties. In: Linzer M (ed) Ultrasonic tissue characterization II. NBS Special Publication 525, U.S. Government Printing Office, Washington, DC pp 19–27
16. Bamber JC (1983) Ultrasonic propagation properties of the breast. In: Jellins J, Kobayashi T (eds) Ultrasonic examination of the breast. John Wiley and Sons, Chichester pp 37–44
17. Jonson R, Manson LG, Rungren Å, Szück J (1990) Dual-photon absorptiometry for determination of bone mineral content in the calcaneus with correction for fat. Phys Med Biol 7:961–969
18. Rossman PJ, Zagzebski JA, Mesina C, Sorenson J, Mazess RB (1989) Comparison of speed of sound and ultrasonic attenuation in the os calcis to photon absorptiometry measurements in the radius, femur and lumbar spine. Clin Phys Physiol Meas 10:353–360
19. Agren M, Karellas A, Leahey D, Marks S, Baran D (1991) Ultrasound attenuation of the calcaneus: a sensitive and specific discriminator of osteopenia in postmenopausal women. Calcif Tissue Int 48:240–244