TITLE PAGE

Title:

Calcaneal ultrasound reference ranges for Australian men and women: The Geelong Osteoporosis Study

Authors:

Haslinda Gould^{1,2}

Sharon L. Brennan^{1,2}

Geoff C. Nicholson³

Mark A. Kotowicz^{1,2,4}

Margaret J. Henry^{1,5}

Julie A. Pasco^{1,2,4}

Affiliations:

¹NorthWest Academic Centre, Department of Medicine, The University of Melbourne, St Albans, Victoria, AUSTRALIA

²School of Medicine, Deakin University, Geelong, Victoria, AUSTRALIA

³Rural Clinical School, The University of Queensland, Toowoomba, Queensland, AUSTRALIA

⁴Department of Medicine, Barwon Health, Geelong, Victoria, AUSTRALIA

⁵Barwon South Western Region Integrated Cancer Services, Barwon Health, Geelong, Victoria, AUSTRALIA

Corresponding author:

Haslinda Gould

Barwon Epidemiology and Biostatistics Unit

School of Medicine

Deakin University

Kitchener House

PO Box 281

GEELONG VIC 3220

T: +61 3 5226 7414

F: +61 3 5246 5165

E: hgould@barwonhealth.org.au

Abstract:

Purpose: To develop calcaneal (heel) ultrasound reference ranges for the Australian adult population using a population-based random sample.

Methods: Men and women aged ≥ 20 yr were randomly selected from the Barwon Statistical Division in 2001-06 and 1993-97, respectively, using the electoral roll. Broadband ultrasound attenuation (BUA), speed of sound (SOS) and stiffness index (SI) were measured at the heel using a Lunar Achilles Ultrasonometer. Gender-specific means and standard deviations for BUA, SOS and SI were calculated for the entire sample (men 20-93 yr, $n=1104$; women 20-92 yr, $n=914$) and for participants aged 20-29 yr (men $n=157$; women $n=151$). Associations between ultrasound measures and age were examined using linear regression.

Results: For men, mean ($\pm$ standard deviation) BUA, SOS and SI were 118.7 ± 15.8 dB/MHz, 1577.0 ± 43.7 m/s and 100.5 ± 20.7 ; values for women were consistently lower (111.0 ± 16.4 dB/MHz, $P<0.001$; 1571.0 ± 39.0 m/s, $P=0.001$; and 93.7 ± 20.3 , $P<0.001$, respectively). BUA was higher in young men compared with young women (124.5 ± 14.4 vs 121.0 ± 15.1 dB/MHz) but SOS (1590.1 ± 43.1 vs 1592.5 ± 35.0 m/s) and SI (108.0 ± 19.9 vs 106.3 ± 17.7) were not. The relationships between age and each ultrasound measure were linear and negative across the age range in men; associations were also negative in women but non-linear.

Conclusion: These data provide reference standards to facilitate the assessment of fracture risk in an Australian population using heel ultrasound.

Keywords:

Calcaneus

Ultrasonography

Reference ranges

Bone and bones

Conflict of interest:

No disclosures.

SPECIFIC REMARKS

Mini abstract:

Heel ultrasound is a more portable modality for assessing fracture risk than dual energy X-ray absorptiometry, and does not use ionising radiation. Fracture risk assessment requires appropriate reference data to enable comparisons. This study reports the first heel ultrasound reference ranges for the Australian population.

TEXT

Introduction

Measuring the quantity and quality of bone is important in the assessment of fracture risk. Calcaneal (heel) ultrasound measures broadband ultrasound attenuation (BUA) and speed of sound (SOS); stiffness index (SI), which is measured by some heel ultrasound devices, is a combination of these parameters. SOS and SI may also be referred to as velocity of sound (VOS) and quantitative ultrasound index (QUI), respectively, depending on the QUS device. BUA and SOS measured at the heel have shown weak to moderate correlation with heel bone mineral density (BMD) [1] and femoral BMD [2] assessed using dual energy X-ray absorptiometry (DXA). Ultrasound parameters are influenced by trabecular structure and organisation [3,4]. Measured at the heel, ultrasound parameters are associated with fracture independently of hip BMD [5,6]; heel ultrasound is also safer, cheaper and more portable than DXA [7]. These characteristics of heel ultrasound help to support the notion of this modality as an alternative or adjunct to DXA for the purpose of fracture risk assessment. A necessary precursor to the use of heel ultrasound in clinical practice, however, is the development of appropriate heel ultrasound reference ranges to facilitate comparisons with age-matched and young adult reference standards.

There are no published heel ultrasound reference ranges for the Australian population and using published normative data developed in other populations in lieu of Australian-specific data may not be appropriate. Furthermore, sampling strategies used to develop reference data provided by the manufacturers of heel ultrasound devices may not be representative or random. The use of inappropriate reference data for the purpose of clinical assessment may bias estimates of fracture risk [8]. The reference data presented here, generated using a Lunar Achilles Insight Ultrasonometer, represent that of a largely homogeneous Caucasian sample of men and women residing in south-eastern Australia.

Methods

Study participants

This study is positioned within the Geelong Osteoporosis Study (GOS), a prospective cohort study initiated in 1993 [9]. Participants in the GOS were men (n=1540) and women (n=1494) aged ≥ 20 yr randomly selected (over the periods 2001-06 and 1993-97, respectively) from a region in south-eastern Australia defined by the Australian Bureau of Statistics (ABS) as the Barwon Statistical Division (BSD). The baseline response was 67% and 77% for the male and female cohorts, respectively. The sampling frame was the Commonwealth of Australia electoral roll, registration for which is compulsory for individuals aged ≥ 18 yr. Age-stratification resulted in approximately 100 participants in each 5 yr age group within the range 20-69 yr and approximately 200 participants aged 70-79 yr and ≥ 80 yr, as previously described [10,9]. A further 221 women aged 20-31 yr were recruited from the BSD during the 10 yr follow-up for women (2004-08) in the same fashion as described above.

BUA, SOS and SI were assessed for the left heel using an Achilles Insight Ultrasonometer (GE Lunar, Madison, WI, USA) at baseline for men, and at the 10 yr follow-up for women.

Measurements were performed by trained personnel using the same QUS device for men and women.

In vivo precision was 4.0%, 0.3% and 3.2% for BUA, SOS and SI, respectively.

Participants were included in analyses if they had complete measures of BUA, SOS, SI and anthropometry. A total of 436 men and 801 women had incomplete data and were excluded leaving 1104 men and 914 women who were included in analyses. There were no other exclusion criteria such as fracture history or exposure to drugs or diseases that affect bone metabolism. Ethical approval for the study was obtained from the Barwon Health Human Research Ethics Committee and all participants provided written consent.

Statistical analyses

Histograms of the heel ultrasound measures, BUA, SOS and SI and the covariates, age, weight and height for men and women were checked for normal distribution. Relationships between heel ultrasound measures and each of age, weight and height were examined using scatter plots. Gender-specific standard deviations and means for BUA, SOS and SI were calculated for the entire study sample and for those aged 20-29 yr. All modelling was performed using linear regression. The independent variables, age, weight and height were centred (by subtracting the appropriate mean from each variable) before including them in linear regression models to attenuate the effect of collinearity. The likelihood ratio test was used as a test for linearity; models for each heel ultrasound measure with and without squared exposure terms were compared to determine if associations were linear or non-linear. All possible regressions involving the dependent and independent variables (and the square transformations of age) were examined. The selection of parsimonious models for BUA, SOS and SI for men and women was a compromise between models with the fewest number of variables, lowest Mallows' Cp statistic, lowest standard error and highest coefficient of determination (R^2). The assumptions of linear regression were examined by visualising the variance and distribution of residuals for models using scatter plots and histograms, respectively. Correlation coefficients for the relationships between both heel ultrasound measures and each covariate were also calculated. Simple comparisons for continuous variables were performed using un-paired two-sample t-tests. The distributions of BUA and SOS in relation to the independent variables, age, weight and height were displayed as scatter plots along with regression lines and 95% prediction intervals; relationships with SI have not been shown. Statistical analyses were carried out using Stata version 11.1 (StataCorp LP, College Station, TX, USA).

Results

Characteristics of the study population

Table 1 presents subject characteristics for the 1104 men aged 20-93 yr and 914 women aged 20-92 yr included in analyses. Median ages of men and women were 56.2 yr (interquartile range [IQR]: 38.4 – 73.5) and 51.9 yr (IQR: 37.2 – 65.6), respectively. On average, men were taller (mean height: 175.1 vs 162.3 cm) and heavier (mean weight: 82.8 vs 72.7 kg) than women. The heel ultrasound measures, BUA, SOS and SI were normally distributed in both men and women. For men, mean ($\pm$ standard deviation) BUA, SOS and SI were 118.7 ± 15.8 dB/MHz, 1577.0 ± 43.7 m/s and 100.5 ± 20.7 ; values for women were consistently lower (111.0 ± 16.4 dB/MHz, $P < 0.001$; 1571.0 ± 39.0 m/s, $P = 0.001$; and 93.7 ± 20.3 , $P < 0.001$; Table 1). BUA was higher in young men ($n = 157$) compared with young women ($n = 151$; 124.5 ± 14.4 vs 121.0 ± 15.1 dB/MHz, $P = 0.036$) but SOS (1590.1 ± 43.1 vs 1592.5 ± 35.0 m/s, $P = 0.589$) and SI (108.0 ± 19.9 vs 106.3 ± 17.7 , $P = 0.423$) were not. Overall, average BUA, SOS and SI for the young adult sample was higher than that for the older sample (122.8 vs 113.9 dB/MHz, 1591.3 vs 1571.3 m/s and 107.2 vs 95.7 ; $P < 0.001$ for all three comparisons).

Univariable associations between age and heel ultrasound measures

The relationships between age and each of BUA, SOS and SI were different for men and women; likelihood ratio tests suggested that the associations between age and ultrasound measures were linear in men and non-linear in women. The relationship between BUA and age in men was linear and negative across the age range examined (Figure 1a and Table 3); BUA decreased by 0.17 dB/MHz (95 % CI: -0.22, -0.12 dB/MHz, $P < 0.001$) per year, however the strength of this association was weak ($r = -0.22$ [95% CI: -0.27, -0.16]). For women, the association between age and BUA was also negative but non-linear as BUA decreased at a greater rate after middle-age (Figure 1b and Table 3). These patterns of association were also observed for SOS and age; SOS decreased linearly by 0.51 m/s (95% CI: -0.63, -0.38 m/s, $P < 0.001$) per year in men (Figure 2a and Table 3) and the

strength of the association was weak ($r=-0.23$ [95% CI: -0.29, -0.17]). In women, the decline in SOS per year was non-linear (Figure 2b and Table 3). The relationship between SI and age was similarly linear (β [95 % CI]: -0.25[-0.31, -0.19] per yr) and weak ($r=-0.24$ [95% CI: -0.30, -0.19]) in men and non-linear in women (Table 3).

Univariable associations between anthropometry and heel ultrasound measures

The association between BUA and weight was linear for both genders but stronger for women (Figures 1e-f). BUA increased by 0.13 dB/MHz per kg increase in weight (95 % CI: 0.07, 0.20 dB/MHz, $P<0.001$) in men and by 0.31 dB/MHz (95 % CI: 0.25, 0.38 dB/MHz, $P<0.001$) in women. The relationship in men was weak ($r=0.12$ [95% CI: 0.06, 0.18]) and weak to moderate in women ($r=0.31$ [95% CI: 0.25, 0.37]). The relationship between BUA and height was positive and linear for both genders (Figures 1c-d), but like that between BUA and weight, the association was stronger in women (β [95 % CI]: 0.85 [0.70, 1.00] dB/MHz per cm, $P<0.001$ vs 0.15 [0.03, 0.28] dB/MHz per cm, $P=0.02$, respectively). As with weight, BUA and height were more highly correlated in women than men ($r=0.35$ [95% CI: 0.29, 0.41] vs $r=0.07$ [95% CI: 0.01, 0.13], respectively). In contrast with BUA, SOS was not associated with weight and height in men (β [95 % CI]: 0.03 [-0.15, 0.21] m/s per kg, $P=0.76$ and -0.15 [-0.50, 0.20] m/s per cm, $P=0.40$, respectively) (Figures 2c and e); however, SOS was positively associated with both in women (β [95 % CI]: 0.36 [0.21, 0.52] m/s per kg, $P<0.001$ and 1.05 [0.68, 1.40] m/s per cm, $P<0.001$, respectively) (Figures 2d and f). In women, the correlations between SOS and each of weight and height (r [95% CI]: 0.15 [0.09-0.21] and 0.18 [0.12-0.24], respectively) were approximately half that of BUA and each of weight and height (r [95% CI]: 0.31 [0.25-0.37] and 0.35 [0.29-0.41], respectively). There were no correlations between SOS and each of weight and height in men ($r=0.01$ [95% CI: -0.05, 0.07] and $r=-0.03$ [95% CI: -0.08, 0.03], respectively). SI was associated with weight and height in women (β [95 % CI]: 0.31 [0.23, 0.39] per kg, $P<0.001$ and 0.86 [0.67, 1.04] per cm, $P<0.001$) but not with height in men (0.10 [0.01,

0.18] per kg, $P=0.025$ and 0.06 [-0.11, 0.23] per cm, $P=0.481$). There was no correlation between SI and height for men (r [95% CI]: 0.02[-0.04, 0.08] and that between SI and weight for men was weak (0.07 [0.01, 0.13]); correlations between SI and each of weight and height in women were weak to moderate (0.25[0.18, 0.31] and 0.29[0.23, 0.34], respectively).

Multivariable models for BUA, SOS and SI

All multivariable models incorporating the covariates of age, weight and height were examined for their ability to predict BUA, SOS and SI in men and women. The regression coefficients for the parsimonious models are shown in Table 3. Models were different for men and women and in the case of the former, models were also different for each ultrasound measure. For men, the models for BUA and SI included the variables, age, weight and height (adjusted R^2 , 6.0 and 7.6%, respectively) but only age and height for SOS (adjusted R^2 , 7.3%), whereas for women, models for BUA, SOS and SI included age (and the square transformation of age) and weight (adjusted R^2 , 37.0, 25.8 and 35.5 % , respectively).

Discussion

This study presents normative heel ultrasound data for a large population-based sample of adult men and women randomly selected from a region in south-eastern Australia. ABS census data has been used to show that the population in the study region (BSD) and the Australian population are comparable in terms of age structure and weekly income [9]. Standardisation of BUA, SOS and SI values using appropriate reference data will facilitate the assessment of fracture risk using heel ultrasound, a more practical and accessible modality for measuring bone quantity and quality than DXA.

Previous studies have developed normative heel ultrasound data for men and women residing in Europe [11-17], Asia [18-20] and South America [21]. Comparing the reference data presented here to that in extant literature is difficult due to the use of different heel ultrasound devices. Also, reference data for BUA, SOS and SI for women and men aged 20-29 yr cannot be directly compared with that for different heel ultrasound measures and age groups. Despite these limitations, there is some evidence to suggest that, notwithstanding differences due to chance and those arising from participant selection strategies adopted, published heel ultrasound normative data are apparently different when developed in different regions (and ethnically different samples) using heel ultrasound devices by the same manufacturer [13,18] and for different ethnic groups in the same population [13]. Also, heel ultrasound parameters measured in the same population using different devices do not correlate perfectly; Njeh et al showed that the correlation coefficients for BUA or SOS measured using 6 different heel ultrasound devices were between 0.69 and 0.93[2]. This suggests that normative heel ultrasound data that are region-, ethnicity- and device-specific are necessary for valid, informative comparisons in a clinical setting. While some studies that used Lunar Achilles ultrasound devices showed trends that were inconsistent across the adult age range, overall, general declines in ultrasound measures with increasing age have been reported for men and women [15,16,20,21]. Young adult reference means for BUA, SOS or SI measured using Lunar Achilles ultrasound devices for 20-29 yr Greek women [15], 25-34 yr Swedish men and women [16] and 20-24 yr Chinese men [20], Brazilian women [21] and UK women [11] appear to be lower than the equivalent data reported here for 20-29 yr Caucasian men and women residing in Australia. Discrepancies between data may be due to the use of different Lunar Achilles ultrasonometers; this study utilised the Lunar Achilles 'Insight' model whereas the studies mentioned above used alternative Lunar Achilles devices or the model used was indeterminable [20].

In addition to generating reference data we also explored the relationship between heel ultrasound measures and each of age, weight and height. We have shown that the association between age and

each of BUA, SOS and SI is negative and appears to be linear in men and non-linear in women. While some studies have reported a constant decline in ultrasound measures with increasing age in both genders others have shown that non-linear models for BUA and a measure equivalent to SOS, VOS, for women [13,17] and men [17] improved R^2 values. This lack of consistency in reported associations between age and ultrasound measures may be due to genetic and lifestyle differences between the study populations or biases arising from participant selection. Consistent with our findings, studies that included both male and female participants showed that the rate of decline in ultrasound measures appeared to be more marked in women [14,16-18]. This trend was independent of physical activity and height as reported by Landin-Wilhelmsen et al and was not consistent across the younger adult age range as reported by Langton et al. Overall we found that age, weight and height were more strongly associated with BUA, SOS and SI in women than men. The reason for this discrepancy between genders may be due to the existence of factors, other than those examined, explaining the variance in BUA and SOS (particularly in men). In a multi-centre study in European men aged 40-79 yr BUA, SOS and a measure equivalent to SI, QUI, were positively associated with variables reflecting physical activity and negatively associated with smoking and the time taken to go from sitting to standing or to walk 50 feet, independently of weight, age and centre [22]. Saadi et al reported a similar relationship between physical activity and each of BUA, SOS and QUI in postmenopausal women residing in the United Arab Emirates after adjusting for age and body mass index (BMI) [19]. Landin-Wilhelmsen et al confirmed this association between physical activity and each of BUA, SOS and SI in both men and women after adjusting for age and/or height [16]. Our findings indicate that age and anthropometry alone do not account for all of the variation in BUA, SOS and SI; for women, 37%, 26% and 36% of the variance in BUA, SOS and SI, respectively, was explained by age and weight. The contribution of lifestyle-related factors such as physical activity has not been addressed in this study. Other studies that examined similar relationships also provide evidence of this; R^2 values for models that included a combination of age, weight, height, years since

menopause or BMI for women were 19% and 44% for SI [21,15], 33% for BUA [21] and 45% for SOS [21].

Ultrasound measures have been identified as risk factors for fracture, independently of BMD [5,6]; in contrast, Jokinen et al reported that BMI and physical performance were better predictors of hip fracture in their cohort of elderly women than BMD and heel ultrasound [23]. Current fracture risk algorithms do not incorporate ultrasound measures [24-26]. Considering that access to DXA is limited in some populations and that the cost of fracture in both human- [27] and economic-terms [28] is significant, the relative performance of models of fracture risk that include ultrasound measures alone or in combination with BMD should be explored.

The mean and standard deviation of BUA, SOS and SI for the sample of Australian men and women in this study are appropriate estimates of the population values of these heel ultrasound parameters as the sampling strategy adopted in this study was random and representative. The method of participant selection for the purpose of generating reference data can bias standardised values [8]. The importance of a large representative random sample in the development of reference ranges for BMD has been recognised by the World Health Organisation [29]. While the reference data presented here were developed in a random and representative sample, the incomplete response at baseline and the loss to follow-up in the female cohort means that it is possible that our study sample may be a healthier subset of the adult population. Also, considering that at least 99% of the male and female cohorts at baseline of the GOS were Caucasian [8,30] these data may not be appropriate for the assessment of fracture risk in other ethnic groups.

To the best of our knowledge, this study provides the first heel ultrasound reference ranges for BUA, SOS and SI in a representative sample of the Australian population. These data facilitate the comparison of BUA, SOS and SI with age-matched and young adult reference standards in a clinical setting. Further work is required to determine whether these data are applicable to non-Caucasian sections of the Australian population and to improve the consistency of reporting of heel ultrasound reference data.

Acknowledgements

The study was funded by the National Health and Medical Research Council (NHMRC), Perpetual Trustees and Geelong Region Medical Research Foundation. Dr Sharon Brennan is the recipient of an NHMRC Early Career Fellowship (1012472).

References

1. Bouxsein ML, Radloff S, Radloff SE (1997) Quantitative ultrasound of the calcaneus reflects the mechanical properties of calcaneal trabecular bone. *J Bone Miner Res* 12 (5):839-846
2. Njeh CF, Hans D, Li J, Fan B, Fuerst T, He YQ, Tsuda-Futami E, Lu Y, Wu CY, Genant HK (2000) Comparison of six calcaneal quantitative ultrasound devices: precision and hip fracture discrimination. *Osteoporos Int* 11 (12):1051-1062
3. Glüer CC, Wu CY, Genant HK (1993) Broadband ultrasound attenuation signals depend on trabecular orientation: an in vitro study. *Osteoporos Int* 3 (4):185-191
4. van den Bergh JPW, van Lenthe GH, Hermus ARMM, Corstens FHM, Smals AGH, Huiskes R (2000) Speed of sound reflects Young's modulus as assessed by microstructural finite element analysis. *Bone* 26 (5):519-524
5. Bauer DC, Gler CC, Cauley JA, Vogt TM, Ensrud KE, Genant HK, Black DM (1997) Broadband ultrasound attenuation predicts fractures strongly and independently of densitometry in older women. A prospective study. Study of Osteoporotic Fractures Research Group. *Arch Intern Med* 157 (6):629-634
6. Moayyeri A, Kaptoge S, Dalzell N, Luben R, Wareham N, Bingham S (2009) The effect of including quantitative heel ultrasound in models for estimation of 10-year absolute risk of fracture. *Bone* 45 (2):180-184

7. Gluer CC (1997) Quantitative ultrasound techniques for the assessment of osteoporosis: expert agreement on current status. The International Quantitative Ultrasound Consensus Group. *J Bone Miner Res* 12 (8):1280-1288
8. Henry MJ, Pasco JA, Seeman E, Nicholson GC, Sanders KM, Kotowicz MA (2001) Assessment of fracture risk: value of random population-based samples--the Geelong Osteoporosis Study. *J Clin Densitom* 4 (4):283-289
9. Pasco J, Nicholson G, Kotowicz M (2011) Cohort profile: Geelong Osteoporosis Study. *International Journal of Epidemiology* IN PRESS.
10. Henry MJ, Pasco JA, Nicholson GC, Seeman E, Kotowicz MA (2000) Prevalence of osteoporosis in Australian women: Geelong Osteoporosis Study. *J Clin Densitom* 3 (3):261-268
11. Truscott J (1997) Reference data for ultrasonic bone measurement: variation with age in 2087 Caucasian women aged 16-93 years. *Br J Radiol* 70 (838):1010-1016
12. Kastelan D, Kujundzic-Tiljak M, Kraljevic I, Kardum I, Giljevic Z, Korsic M (2006) Calcaneus ultrasound in males: Normative data in the Croatian population (ECUM study). *J Endocrinol Invest* 29:221-225
13. Hinkley HJ, Drysdale IP, Walters NJ, Bird D (2004) Normative data for ultrasound measurement of the calcaneus within different female ethnic groups. *Br J Radiol* 77 (921):740-744
14. Sosa M, Saavedra P, Munoz-Torres M, Alegre J, Gomez C, Gonzalez-Macias J (2002) Quantitative ultrasound calcaneus measurements: Normative data and precision in the Spanish population. *Osteoporos Int* 13 (6):487-492
15. Trovas G, Tsekoura M, Galanos A, Dionyssiotis Y, Dontas I, Lyritis G, Papaioanou N (2009) Quantitative ultrasound of the calcaneus in greek women: normative data are different from the manufacturer's normal range. *J Clin Densitom* 12 (3):353-359

16. Landin-Wilhelmsen K, Johansson S, Rosengren A, Dotevall A, Lappas G, Bengtsson B, Bengtsson BA, Wilhelmsen L (2000) Calcaneal ultrasound measurements are determined by age and physical activity. Studies in two Swedish random population samples. *J Intern Med* 247 (2):269-278
17. Langton C, Langton D (1997) Male and female normative data for ultrasound measurement of the calcaneus within the UK adult population. *Br J Radiol* 70 (834):580-585
18. Lim YW, Chan L, Lam KS (2005) Broadband ultrasound attenuation reference database for Southeastern Asian males and females. *Ann Acad Med Singapore* 34 (9 Supp):545-547
19. Saadi HF, Reed RL, Carter AO, Dunn EV, Qazaq HS, Al-Suhaili AR (2003) Quantitative ultrasound of the calcaneus in arabian women: relation to anthropometric and lifestyle factors. *Maturitas* 44 (3):215-223
20. Zhu Z-Q, Liu W, Xu C, Han S, Zu S, Zhu G, Liu W, Xu C-L, Han S-M, Zu S-Y, Zhu G-J (2008) Reference data for quantitative ultrasound values of calcaneus in 2927 healthy Chinese men. *J Bone Miner Metab* 26 (2):165-171
21. Helder de Moura Castro C, Medeiros Pinheiro M, Lúcia Szejnfeld V (2000) Quantitative ultrasound of the calcaneus in Brazilian Caucasian women: normative data are similar to the manufacturer's normal range. *Osteoporos Int* 11 (11):923-928
22. Pye S, Devakumar V, Boonen S, Borghs H, Vanderschueren D, Adams J (2010) Influence of lifestyle factors on quantitative heel ultrasound measurements in middle-aged and elderly men. *Calcif Tissue Int* 86 (3):211-219
23. Jokinen H, Pulkkinen P, Korpelainen J, Heikkinen J, Keinänen-Kiukkaanniemi S, Jamsa T (2010) Risk factors for cervical and trochanteric hip fractures in elderly women: A population-based 10-Year follow-up study. *Calcif Tissue Int* 87 (1):44-51
24. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E (2008) FRAX and the assessment of fracture probability in men and women from the UK. *Osteoporos Int* 19 (4):385-397

25. Henry MJ, Pasco JA, Sanders KM, Nicholson GC, Kotowicz MA (2006) Fracture Risk (FRISK) Score: Geelong Osteoporosis Study. *Radiology* 241 (1):190-196
26. Nguyen ND, Frost SA, Center JR, Eisman JA, Nguyen TV (2008) Development of prognostic nomograms for individualizing 5-year and 10-year fracture risks. *Osteoporos Int* 19:1431-1444
27. Pasco JA, Sanders KM, Hoekstra FM, Henry MJ, Nicholson GC, Kotowicz MA (2005) The human cost of fracture. *Osteoporos Int* 16 (12):2046-2052
28. Randell A, Sambrook PN, Nguyen TV, Lapsley H, Jones G, Kelly PJ, Eisman JA (1995) Direct clinical and welfare costs of osteoporotic fractures in elderly men and women. *Osteoporos Int* 5 (6):427-432
29. World Health Organisation (2003) WHO Scientific Group on Prevention and Management of Osteoporosis. *World Health Organ Tech Rep Ser* (921):1-192
30. Henry MJ, Pasco JA, Korn S, Gibson JE, Kotowicz MA, Nicholson GC (2010) Bone mineral density reference ranges for Australian men: Geelong Osteoporosis Study. *Osteoporos Int* 21 (6):909-917

Table 1: Characteristics of the sample of men and women used to generate normative heel ultrasound data.

	Men	Women
	(n=1104)	(n=914)
Age (yr), (median [IQR])	56.2 (38.4 – 73.5)	51.9 (37.2 – 65.6)
Weight (kg), (mean $\pm$ SD)	82.8 $\pm$ 14.4	72.7 $\pm$ 16.1
Height (cm), (mean $\pm$ SD)	175.1 $\pm$ 7.4	162.3 $\pm$ 6.8

Note: IQR = interquartile range, SD = standard deviation

Table 2: Broadband ultrasound attenuation (BUA; dB/MHz), speed of sound (SOS; m/s) and stiffness index (SI; %) by gender and ten-year age group and for the entire age range, 20-93 yr for men and 20-92 yr for women (displayed as mean $\pm$ deviation)

		Age group (yr)							
		20-29	30-39	40-49	50-59	60-69	70-79	+80	Total
Men	BUA	124.50 $\pm$ 14.39	122.41 $\pm$ 16.73	120.34 $\pm$ 14.51	118.03 $\pm$ 13.66	116.16 $\pm$ 14.93	116.09 $\pm$ 15.65	114.25 $\pm$ 17.95	118.70 $\pm$ 15.65
	SOS	1590.07 $\pm$ 43.07	1589.15 $\pm$ 43.52	1584.95 $\pm$ 41.29	1576.60 $\pm$ 41.62	1573.28 $\pm$ 42.20	1566.28 $\pm$ 41.01	1562.41 $\pm$ 45.82	1577.04 $\pm$ 41.01

	SI	108.03 ± 19.91	106.37 ± 21.42	103.87 ± 19.36	100.00 ±18.67	97.79 ±19.71	95.83±19.87	93.51±22.26	100.54±
	n	157	145	141	162	160	186	153	110
Women	BUA	120.96 ± 15.06	118.39 ± 13.66	117.41 ±13.59	112.33 ±13.13	104.04 ±11.74	99.71±14.19	88.45±14.05	111.01±
	SOS	1592.50 ± 34.96	1590.71 ± 34.87	1581.62 ± 36.24	1571.36 ±30.49	1558.24 ±30.95	1547.07±37.73	1524.08±30.61	1571.00±
	SI	106.30 ± 17.73	104.14 ± 17.10	100.95 ± 17.00	94.72 ±15.71	85.54 ±14.96	79.56±18.47	65.67±15.51	93.73±2
	n	151	116	158	168	146	122	53	914

Table 3: Regression coefficients for (a) best-fitting models including age (yr) only for BUA, SOS and SI ; and (b) parsimonious models for BUA,SOS and SI incorporating one or more of the independent variables, age, weight (kg) or height (cm)

		Constant	(age-c) [*]	(age-c) ^{2*}	(weight-c) [*]	(height-c) [*]	Adjusted R ² (%)
(a)	Men						
	BUA	118.70	-0.17	-	-	-	4.6
	SOS	1577.04	-0.51	-	-	-	5.2
	SI	100.54	-0.25	-	-	-	5.9
	Women						
	BUA	113.73	-0.48	-0.01	-	-	31.9
	SOS	1575.96	-1.03	-0.02	-	-	25.2

	SI	96.92	-0.60	-0.01	-	-	32.9
(b)	Men						
	BUA	118.70	-0.19	-	0.15	-0.21	6.0
	SOS	1577.04	-0.67	-	-	-0.96	7.3
	SI	100.54	-0.32	-	0.15	-0.45	7.6
	Women						
	BUA	113.08	-0.46	-0.01	0.24	-	37.0
	SOS	1575.43	-1.01	-0.01	0.19	-	25.8
	SI	96.34	-0.59	-0.01	0.21	-	35.5

Note: BUA = broadband ultrasound attenuation (dB/MHz), SOS = speed of sound (m/s)

* the mean age, weight or height for men and women was subtracted from these respective variables to give 'age-c', 'weight-c' and 'height-c' (i.e. 55.62 yr, 82.84 kg and 175.06 cm for men and 51.76 yr, 72.65 kg and 162.27 cm for women)

Men

Women

Figure 1a-f: Associations between broadband ultrasound attenuation (BUA) and each of age, height and weight for men and women. Regression lines and 95 % prediction intervals are shown. Figure 1e is truncated at 150 kg (n=2 men excluded) to allow clearer visualisation of the spread of the data.

Men

Women

Figure 2a-f: Associations between speed of sound (SOS) and each of age, height and weight for men and women. Regression lines and 95 % prediction intervals are shown. Figure 2e is truncated at 150 kg (n=2 men excluded) to allow clearer visualisation of the spread of the data.