Determinants of age-associated changes in os calcis ultrasonic indices in elderly women: potential involvement of geriatric hyposomatotropism in bone fragility

STEVEN BOONEN, PATRICK H. F. NICHOLSON, GEERT LOWET, XIAO GUANG CHENG, GEERT VERBEKE, EMMANUEL LESAFFRE, JEROEN AERSSENS, JAN DEQUEKER

1Arthritis and Metabolic Bone Disease Research Unit and 2Division of Biomechanics and Engineering Design, Katholieke Universiteit Leuven, Leuven, Belgium
3Department of Internal Medicine, Division of Geriatric Medicine, UZ Leuven, Brusselsestraat 69, B-3000 Leuven, Belgium
4Department of Epidemiology, Biostatistical Centre, Katholieke Universiteit Leuven, Leuven, Belgium

Address correspondence to: S. Boonen. Fax: (+32) 16 337941.

Abstract

Objective: ultrasound measures a clinically relevant property of bone strength in addition to and distinct from bone mass. The aim of the present study was to examine the effects of healthy ageing on ultrasound measurements of the calcaneus.

Design: cross-sectional study.

Study participants: a sample of 177 community-dwelling healthy women aged 70–87 years. Exclusion criteria were diseases or medications known to affect the musculoskeletal system or the somatotrophic axis.

Measurements: serum levels of 1,25-dihydroxyvitamin D$_3$ and insulin-like growth factor-I (IGF-I) were measured by radioimmunoassay, serum 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) was determined by competitive binding assay and serum parathyroid hormone was assessed immunochemically. Isometric and isokinetic quadriceps strength were evaluated using a Cybex II system. Calcaneal ultrasound indices—broadband ultrasound attenuation (BUA) and speed of sound (SOS)—were measured with an Achilles system.

Results: we found a significant decrease with ageing in BUA and SOS (—0.5 and —1.3% per year, respectively), suggesting a continuing loss of bone quality. Quadriceps strength, serum IGF-I and 25(OH)D$_3$ constituted the best predictors of BUA, while IGF-I was the only parameter found to be independently associated with SOS.

Conclusion: these findings suggest that, among other factors, the activity of the growth hormone-IGF-I axis is of importance for skeletal integrity. Age-related bone fragility may, in part, be related to geriatric hyposomatotropism.

Keywords: bone quality, broadband ultrasound attenuation, insulin-like growth factor-I, ultrasound, speed of sound

Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture [1]. Fractures of the proximal femur in particular are associated with significant morbidity and mortality in the elderly [2]. In order to allow targeting of preventive care, there is considerable interest in predicting fracture risk [3–9]. Bone densitometry is an established method to detect incipient bone fragility and to determine the risk of developing fractures [3, 4]. The most commonly used method for assessing bone mass is dual-energy x-ray absorptiometry, which is a precise method for the determination of bone mineral density (BMD) at the proximal femur [5]. However, numerous in vitro studies have indicated that bone strength is only partially related to bone density [6, 7]. Densitometry performance has improved to the point where the inability of BMD
readings to account completely for mechanical failure can no longer be attributed to measurement variance. It is clear, therefore, that qualitative factors beyond reduced density contribute to bone fragility. Aspects of bone quality, such as bone microstructure [8] and accumulation of microdamage [9] have been implicated in the pathogenesis of hip fractures, although the extent to which most of these phenomena act independently of bone mass remains uncertain.

Recently, quantitative ultrasound (QUS) has been proposed as an adjunct or alternative to x-ray-based bone densitometry as it does not involve exposure to ionizing radiation and is relatively simple to implement and process [10]. As a mechanical wave, ultrasound interacts with bone in a fundamentally different way from ionizing electromagnetic radiation. In view of the modest correlations between ultrasound and densitometry parameters [11-14], ultrasound may be sensitive to some aspect of bone structure other than bone density. There is mounting evidence from in vitro experiences that attenuation of ultrasound signals may represent a measure of trabecular microarchitecture [12, 15, 16], providing a non-invasive index of bone quality.

With current technology, assessment of skeletal status by ultrasound can be made only at distal sites such as the os calcis, not at the proximal femur. However, there is a rationale for measuring the os calcis, a site comprised primarily of cancellous bone. In a large prospective study, Cummings et al. showed that femoral neck BMD is a significantly better predictor of hip fracture than spine ($P < 0.001$) and radius BMD ($P < 0.002$) [3]. Although femoral neck BMD seemed to have a higher predictive value for hip fracture than os calcis BMD, this was not statistically significant ($P = 0.10$), suggesting that the os calcis is a useful site in general for assessment of osteoporosis. Moreover, several studies have shown the ability of calcaneal QUS [broadband ultrasound attenuation (BUA) and speed of sound (SOS)] to distinguish accurately between individuals with and without fragility fractures, including fractures of the proximal femur [11-14]. Ultrasonic measurements made at the os calcis discriminated between fracture cases and elderly controls as well as dual-energy x-ray absorptiometry and independently of bone density [13, 14]. In addition, at least one study has documented QUS measurement of the calcaneus to be predictive of hip fracture [17].

The mechanisms leading to bone loss in a healthy ageing population remain poorly defined [18]. As BUA and SOS reflect aspects of bone strength that are independent of bone density, studies relating biochemical parameters of bone metabolism and ultrasound might reveal predictors of bone quality beyond those obtainable from density measurements. However, research on determinants of SOS and BUA has been limited, mainly focusing on age and anthropometric indices [19]. Moreover, strictly defined reference QUS data for elderly women have not yet been reported, despite the fact that women over the age of 70 are most at risk for osteoporotic fractures [20].

The purpose of the present cross-sectional analysis was to provide reference intervals for ultrasound variables in ageing women and to evaluate the effect of bone and mineral regulatory hormones, correcting for potential confounding variables with multiple regression. To this end, we measured calcaneal ultrasound parameters and potential determinants of skeletal integrity in a well-defined community-based sample of skeletally healthy older women.

### Subjects and methods

#### Study population

Volunteers were recruited from 50 general practices in the province of Brabant, Belgium. Women were eligible to be included in the study if they were over 70 years of age, non-institutionalized, functionally independent and free from diseases or medications known to affect the musculoskeletal system.

Subjects were excluded if they met any of the following criteria: (i) metabolic bone disease; (ii) rheumatoid arthritis and/or (physician-diagnosed) osteoarthritis; (iii) ischaemic and/or valvular heart disease; (iv) chronic bronchitis and/or emphysema; (v) cerebrovascular disease, at any time, affecting the relevant limb; (vi) non-arthroscopic joint surgery, ever, in the relevant limb; (vii) diabetes, whether controlled or uncontrolled; (viii) thyroid disease, whether controlled or uncontrolled; (ix) body mass index $\geq 30$ kg/m$^2$; (x) systolic blood pressure $\geq 200$ mmHg and/or diastolic pressure $\geq 100$ mmHg; (xi) daily medication, including use of calcium, fluoride or vitamin D supplements; or (xii) ever having used thiazides, glucocorticoids (25 mg prednisone or equivalent per day), oestrogens (or oestrogen-related drugs), anabolic steroids or calcitonins for more than 3 months.

Subjects meeting all criteria were referred to the Arthritis and Metabolic Bone Disease Research Unit. After reassessing the exclusion criteria at the research unit, all tests were performed on the same day and in a set order. A total of 177 independent, healthy volunteers were included, aged 70-87 years. Informed consent was obtained from all the women, and all procedures were approved by the institutional ethical committee.

#### Interview

Study participants answered interviewer-administered questionnaires. A standardized interview [21] was used to obtain basic demographic variables, social and lifestyle characteristics and medical information. Dietary
Ultrasonic determination of changes in os calcis in elderly women

Assessment was based on a frequency questionnaire for calcium intake [22].

Anthropometric measures
Anthropometric measurements were made of height and body weight. Body mass index was calculated as body weight divided by height squared (kg/m²).

Biochemical measurements
Fasting blood samples were collected from all subjects. Total serum calcium, inorganic phosphate, albumin and creatinine were determined by standard analytic methods. Creatinine clearance was estimated according to Cockcroft and Gault, relying on serum creatinine, weight and age [23]. Calcidiol (25-hydroxyvitamin D₃; 25(OH)D₃) was measured by competitive binding assay, calcitriol (1,25-dihydroxyvitamin D₃; 1,25(OH)₂D₃) by radioimmunoassay and vitamin D binding protein (DBP) by single radial immunodiffusion. Details of methodology and validation have been previously reported [24-26]. The free 1,25(OH)₂D₃ index was calculated as the molar ratio of total 1,25(OH)₂D₃ to DBP [27]. Serum intact parathyroid hormone [PTH(1-84)] was measured by a two-step immunochemical method, involving an amino-terminal capture and a mid-regional detecting antibody, as described previously [28]. Insulin-like growth factor-I (IGF-I) was measured by a previously reported radioimmunoassay after acid–ethanol extraction [29].

Muscle strength assessment
Isometric and isokinetic strength was evaluated in the knee extensors of the right leg, primarily the quadriceps, to correspond to the side of calcaneal ultrasound measurement. Strength was measured using an isokinetic dynamometer (Cybex II, Lumex Inc., Ronkonkoma, NY, USA) according to the standardized procedures from the manufacturer. All tests were demonstrated by the assessor before being performed by the volunteer. Maximum isokinetic strength was measured at two angular velocities (60°/s and 90°/s), the highest value of three measurements taken as maximum isokinetic strength for each angular velocity. Quadriceps maximum isokinetic strength was determined as the average of the maximum strength at the different angular velocities. Maximum isometric strength was measured at different angles (90° and 60°) as the highest value of three attempts. Quadriceps maximum isometric strength was determined as the average of the maximum strength at different angles. To determine the short-term reproducibility for each isokinetic velocity and for isometric strength, duplicate measurements (with a minimum interval of 1 h) were performed in a random sample of 20 subjects with a mean age of 76 years. Assessed in this manner, the coefficients of variation ranged from 2.2 to 2.8%. As there was a strong correlation between maximum isokinetic and maximum isometric strength (r = 0.77, P < 0.001), an overall index of quadriceps strength was determined as the mean of these two values.

Ultrasound measurements
Calcaneal ultrasound attenuation was measured with the Achilles system (Lunar Inc., Madison, WI, USA) on the right side. The system consists of two transducers, one acting as the transmitter and the other as the receiver, with the ultrasound beam propagating laterally through the centre of the os calcis. A sequence of discrete pulses (100–600 kHz; centre frequency, 500 kHz), is used and BUA—the rate of loss of ultrasonic intensity with frequency, resulting from the interaction of ultrasound in the medium in which its propagates—measured in dB/MHz using Fourier transformation of the recorded signal. SOS—the velocity of ultrasound transmission through bone—is measured in m/s from the sound propagation time between the transducers. To establish the short-term precision of the method in this population, duplicate measurements (with interim repositioning) were performed in 20 randomly selected cohort members. The in vivo coefficients of variation calculated from these measurements were 1.2 and 0.3% for BUA and SOS, respectively.

Statistical analysis
The relationship between the biochemical parameters was evaluated by calculating Pearson's product moment correlation coefficients. In view of the fact that no normalizing transformation was found for age, Spearman rank correlation was used to assess the effect of age on the biochemical variables. A percentage apparent loss per year was calculated by dividing the slope of the regression line by the mean value. On theoretical grounds SOS cannot be smaller than 1400 m/s. To calculate a clinically meaningful apparent rate of loss that can be compared to the apparent rates of loss of other measurements, we therefore subtracted 1400 m/s from the SOS [19].

The relationship between BUA and SOS was studied by a Pearson correlation analysis. To evaluate the linear relationship between ultrasound parameters and potential determinants of bone quality, univariate regression analyses were performed. Subsequently, multiple regression models were constructed with BUA or SOS as response and age, years since menopause, body mass index, calcium intake, quadriceps strength, 25(OH)D₃, PTH and IGF-I as regressors. No variable selection was applied. The appropriate scale of the response and regressors was evaluated with the program R-code [30].

All other statistical analyses were conducted with the use of SAS (Statistical Analysis Systems Inc., Cary, NC, USA). Reported P values are two-sided. The nominal significance level was set at 0.05.
Table 1. Subject characteristics, biochemical parameters and results of quadriceps and ultrasound measurements

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>74.8</td>
<td>4.4</td>
<td>70-87</td>
</tr>
<tr>
<td>Time since menopause (years)</td>
<td>25.9</td>
<td>6.9</td>
<td>21-52</td>
</tr>
<tr>
<td>Height (kg)</td>
<td>156.3</td>
<td>6.1</td>
<td>137-171</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.3</td>
<td>11.3</td>
<td>38-72</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>810.3</td>
<td>412.1</td>
<td>316-2766</td>
</tr>
<tr>
<td>Total calcium (mg/l00ml)</td>
<td>9.8</td>
<td>0.3</td>
<td>8.9-11.6</td>
</tr>
<tr>
<td>Phosphate (mg/100 ml)</td>
<td>3.0</td>
<td>0.4</td>
<td>2.1-4.8</td>
</tr>
<tr>
<td>Albumin (g/100 ml)</td>
<td>4.3</td>
<td>0.2</td>
<td>3.4-5.0</td>
</tr>
<tr>
<td>Creatinine (mg/100 ml)</td>
<td>1.0</td>
<td>0.2</td>
<td>0.2-1.4</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>50.7</td>
<td>16.5</td>
<td>26-96</td>
</tr>
<tr>
<td>25(OH)D3 (nmol/l)</td>
<td>57.4</td>
<td>29.4</td>
<td>7.1-240.2</td>
</tr>
<tr>
<td>Total 1,25(OH)2D3 (pg/ml)</td>
<td>51.1</td>
<td>12.8</td>
<td>15.9-84.6</td>
</tr>
<tr>
<td>DBP (µg/ml)</td>
<td>341.1</td>
<td>40.9</td>
<td>242.9-467.4</td>
</tr>
<tr>
<td>Free 1,25(OH)2D3 index</td>
<td>2.1</td>
<td>0.5</td>
<td>0.5-3.4</td>
</tr>
<tr>
<td>PTH(1-84) (pg/ml)</td>
<td>14.5</td>
<td>9.5</td>
<td>3-65</td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>88.8</td>
<td>36.9</td>
<td>8.0-205.3</td>
</tr>
<tr>
<td>Quadriceps strength index (Nm)</td>
<td>70.9</td>
<td>20.3</td>
<td>38-98</td>
</tr>
<tr>
<td>BUA (dB/MHz)</td>
<td>98.5</td>
<td>9.9</td>
<td>72-124</td>
</tr>
<tr>
<td>SOS (m/s)</td>
<td>1501.9</td>
<td>25.9</td>
<td>1441-1573</td>
</tr>
</tbody>
</table>

SD, standard deviation; 25(OH)D3, 25-hydroxyvitamin D3; 1,25(OH)2D3, 1,25-dihydroxyvitamin D3; DBP, vitamin D binding protein; PTH(1-84), parathyroid hormone; IGF-I, insulin-like growth factor-I; BUA, broadband ultrasound attenuation; SOS, speed of sound.

*Reference ranges: total calcium, 9.0-10.6 mg/100 ml; phosphate, 2.5-4.5 mg/100 ml; albumin, 3.85-4.75 g/100 ml; creatinine, 0.55-1.1 mg/100 ml; 25(OH)D3, 25-150 nmol/l; total 1,25(OH)2D3, 20-80 pg/ml; PTH(1-84), 3-40 pg/ml; IGF-I, 100-300 µg/L.

Results

Subject characteristics, biochemical data and the results of the quadriceps strength and ultrasound measurements are shown in Table 1.

Biochemical parameters of bone metabolism

Age inversely correlated with calculated creatinine clearance ($r = -0.47$, $P < 0.001$). Serum IGF-I ($r = -0.21$, $P < 0.01$; Figure 1) and 25(OH)D3 ($r = -0.17$, $P < 0.05$) concentrations also decreased as a function of age, whereas serum levels of PTH(1-84) increased ($r = 0.21$, $P < 0.01$). As expected, the serum level of PTH was negatively correlated with 25(OH)D3 ($r = -0.24$, $P < 0.001$), even after adjusting for age, serum calcium and creatinine clearance. Serum levels of total and free 1,25(OH)2D3 were positively correlated with calculated creatinine clearance ($r = 0.16$, $P < 0.05$ and $r = 0.21$, $P < 0.01$, respectively).

Relationship between BUA and SOS

Both ultrasound parameters were found to be significantly interrelated ($r = 0.61$, $P < 0.01$).

Determinants of ultrasound parameters at the os calcis

Univariate analyses

BUA and SOS were negatively correlated with age (Table 2 and Figure 2). Unadjusted estimates by linear regression analysis suggested annual decreases of 0.5 and 1.3% for BUA and SOS, respectively. Body mass...
Ultrasonic determination of changes in os calcis in elderly women

Table 2. Univariate analyses: regression coefficients of potential determinants of bone quality on ultrasound parameters*  

<table>
<thead>
<tr>
<th></th>
<th>Broadband ultrasound attenuation</th>
<th>Speed of sound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta )</td>
<td>SE(( \beta ))</td>
</tr>
<tr>
<td>Age</td>
<td>-0.50</td>
<td>0.16</td>
</tr>
<tr>
<td>Time since menopause (years)</td>
<td>-0.24</td>
<td>0.11</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.38</td>
<td>0.18</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>1.34</td>
<td>1.31</td>
</tr>
<tr>
<td>Quadriceps strength</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td>25(OH)D(_3)</td>
<td>3.19</td>
<td>1.31</td>
</tr>
<tr>
<td>Total 1,25(OH)(_2)D(_3)</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Free 1,25(OH)(_2)D(_3)</td>
<td>0.98</td>
<td>1.45</td>
</tr>
<tr>
<td>PTH(1-84)</td>
<td>-0.09</td>
<td>1.05</td>
</tr>
<tr>
<td>IGF-I</td>
<td>0.05</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Based on log-transformation of calcium intake, 25(OH)D\(_3\) and PTH(1-84).

\( \beta \), regression coefficient; SE(\( \beta \)), standard error; \( R^2 \), percentage of the variance of broadband ultrasound attenuation or speed of sound accounted for by a particular variable; \( P \), two-sided \( P \) value; 25(OH)D\(_3\), 25-hydroxyvitamin D\(_3\); 1,25(OH)\(_2\)D\(_3\), 1,25-dihydroxyvitamin D\(_3\); PTH(1-84), parathyroid hormone; IGF-I, insulin-like growth factor-I; NS, not significant.

index and quadriceps strength were positively related to BUA but not to SOS. Serum 25(OH)D\(_3\) and IGF-I, on the other hand, showed statistically significant positive relations with both BUA and SOS. Calcium intake, 1,25(OH)\(_2\)D\(_3\) (total and free) and PTH(1-84) were not related to any ultrasound parameter.

Multivariate analyses

The independent relations between calcaneal ultrasound and potential determinants of bone quality were tested in multivariate regression analysis and are shown in Table 3. Age was not significant after controlling for other covariables. Quadriceps strength, serum IGF-I and 25(OH)D\(_3\) constituted the best predictors of BUA, while IGF-I was the only parameter found to be independently associated with SOS. However, only a small proportion of the total variance could be explained, with \( R^2 \) ranging from 16.8% for BUA to 17.5% for SOS. Including (total or free) 1,25(OH)\(_2\)D\(_3\) did not improve the model precision (\( P = 0.12 \) and \( P = 0.18 \), respectively).

Discussion

These findings confirm and extend previous observations, indicating a significant age-associated decrease in both

Figure 2. Age-associated changes in (a) broadband ultrasound attenuation [BUA (\( r = -0.47, P < 0.01 \))] and (b) speed of sound [SOS (\( r = -0.48, P < 0.01 \))].
### Table 3. Prediction of ultrasound parameters by multiple regression

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β</th>
<th>SE(β)</th>
<th>P</th>
<th>( R^2_p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Broadband ultrasound attenuation</strong> (( R^2 = 16.8% ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadriceps strength</td>
<td>0.08</td>
<td>0.004</td>
<td>0.03</td>
<td>3.1%</td>
</tr>
<tr>
<td>IGF-I</td>
<td>0.04</td>
<td>0.002</td>
<td>0.04</td>
<td>3.1%</td>
</tr>
<tr>
<td>25(OH)D₃</td>
<td>2.81</td>
<td>1.39</td>
<td>0.04</td>
<td>2.9%</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.23</td>
<td>0.20</td>
<td>NS</td>
<td>1.0%</td>
</tr>
<tr>
<td>PTH(1-84)</td>
<td>-1.17</td>
<td>1.06</td>
<td>NS</td>
<td>0.8%</td>
</tr>
<tr>
<td>Age</td>
<td>-0.24</td>
<td>0.25</td>
<td>NS</td>
<td>0.7%</td>
</tr>
<tr>
<td>Time since menopause</td>
<td>-0.13</td>
<td>0.15</td>
<td>NS</td>
<td>0.6%</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.03</td>
<td>1.41</td>
<td>NS</td>
<td>0.1%</td>
</tr>
<tr>
<td><strong>Speed of sound</strong> (( R^2 = 17.5% ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I</td>
<td>0.18</td>
<td>-2.54</td>
<td>0.01</td>
<td>7.5%</td>
</tr>
<tr>
<td>25(OH)D₃</td>
<td>0.05</td>
<td>2.77</td>
<td>NS</td>
<td>1.6%</td>
</tr>
<tr>
<td>Age</td>
<td>5.50</td>
<td>-3.14</td>
<td>NS</td>
<td>1.4%</td>
</tr>
<tr>
<td>Quadriceps strength</td>
<td>3.62</td>
<td>3.67</td>
<td>NS</td>
<td>1.0%</td>
</tr>
<tr>
<td>PTH(1-84)</td>
<td>-0.92</td>
<td>-0.10</td>
<td>NS</td>
<td>0.6%</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.64</td>
<td>0.40</td>
<td>NS</td>
<td>0.5%</td>
</tr>
<tr>
<td>Time since menopause</td>
<td>0.11</td>
<td>0.10</td>
<td>NS</td>
<td>0.1%</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.10</td>
<td>0.52</td>
<td>NS</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

*Based on log-transformation of calcium intake, 25(OH)D₃ and PTH(1-84).

\( R^2 \), proportion of the variance of broadband ultrasound attenuation or speed of sound accounted for after inclusion of the contributing variables; \( \beta \), regression coefficient; SE(\( \beta \)), standard error; \( P \), two-sided \( P \) value; \( R^2_p \), partial \( R^2 \); 25(OH)D₃, 25-hydroxyvitamin D₃; PTH(1-84), parathyroid hormone; IGF-I, insulin-like growth factor-I; NS, not significant.

BUA and SOS [19, 31, 32]. Yearly percentage reductions for the two parameters were -0.5 and -1.3%, respectively. These rates of decline are in line with those reported by Van Daele *et al.* [19] and suggest a continuing loss of skeletal integrity throughout life. Adjusting for body mass index did not significantly alter the associations between age and ultrasound measurements.

In the concept of age-related osteoporosis, a key role has been attributed to vitamin D deficiency-related secondary hyperparathyroidism, through an increase in bone resorption [33]. A positive relation between 25(OH)D₃ and femoral bone density has indeed been observed, in both middle-aged [34] and elderly [35] women. In keeping with these reports, vitamin D status was found to be independently associated with BUA. In addition, our data confirm the importance of muscle strength as a determinant of bone strength [36, 37] in a normal elderly population, indicating a significant effect of quadriceps strength on BUA. On the other hand, none of these factors was found to be independent predictors of SOS. Our failure to detect an association with either ultrasound parameter suggests that BUA and SOS may reflect different skeletal properties. The fact that the observed correlation (\( r = 0.61 \)) between BUA and SOS, although significant, leaves about 63% of unexplained variability, is consistent with this hypothesis. However, whether the unexplained 63% is related to bone strength or whether it reflects a variability unrelated to osteoporosis status remains to be clarified. Although several studies have demonstrated significant correlations between both ultrasonic parameters and bone strength [38, 39], the relationship between these measurements and the physical and biomechanical properties of heterogeneous materials like bone is extremely complex and substantially unknown [40].

Circulating IGF-I was an independent predictor of both ultrasound parameters, despite the inclusion of established determinants of bone mass. Recently, it has become apparent that with advancing age an increasing proportion of women with no clinical evidence of pituitary pathology show decreases in growth hormone secretion and serum levels of IGF-I [41]. Because growth hormone deficiency [42] and normal ageing [43] are both associated with decreases in bone mass, it is tempting to speculate that geriatric hyposomatropism might account, at least partially, for the age-related increase in bone fragility. The results of this study support the concept that the decreased activity of the growth hormone-IGF-I axis alters bone remodelling. These findings contrast with several cross-sectional attempts that failed to correlate baseline IGF-I, as an integrated measure of growth hormone secretion, with bone mass [44, 45]. In a cross-sectional study involving 57 normal women aged 30-90 (17 of whom were older than 70), Bennet *et al.* found no evidence for an effect of serum IGF-I on lumbar bone density [44]. Similarly, in 18 healthy women aged over...
65 and who were followed over 24 months, changes in spinal and femoral BMD were not related to circulating IGF-I at any time point [45]. However, few older women were included in these studies and skeletal status was assessed by conventional densitometry. Ultrasound may reflect aspects of bone quality that are independent of density and this may explain the observations of the present study.

These conclusions need to be tempered by the limitations of the cross-sectional design used in this study. Although the validity of the findings was enhanced by correction for potential confounding with multiple regression, we acknowledge that confirmation would require longitudinal data. In addition, the factors discussed only accounted for 16.8 and 17.5% of the variance of BUA and SOS, respectively, suggesting that there may be other important unmeasured determinants of QUS yet to be defined. Moreover, our results may not be generalizable since the participants were volunteers and not a random sample of the general elderly population. All studies of human ageing are faced with the problem of defining normality in a population with a high prevalence of chronic disease. In order to study age-related processes, and to provide reference values against which to judge data from elderly patients, disease-free subjects must be selected [46]. This requires tightly defined, pre-determined criteria. Our subjects were not selected as representative of the overall elderly population but to allow us to study the effects of healthy ageing. Finally, our study was not designed to address the extent to which the age-associated changes in ultrasound measurements correspond to an increased susceptibility to fractures. As indicated, there is mounting evidence from numerous studies that ultrasound measures a clinically relevant property of bone quality in addition to and distinct from bone mass. However, it remains to be determined whether the measurement by ultrasound of additional bone properties will enhance fracture-risk prediction compared with radiographic densitometry.

In conclusion, ageing in healthy women is associated with a significant decline in ultrasound measurements of the calcaneus, even after 80 years of age, suggesting a continuing loss of bone quality. According to multiple regression, quadriceps strength, serum IGF-I and 25(OH)D3 constituted the best predictors of BUA, while IGF-I was the only parameter found to be independently associated with SOS. The independent effect of serum IGF-I in addition to that of established bone mass determinants suggests that, among other factors, the activity of the growth hormone-IGF-I axis may be of importance for skeletal integrity.

Acknowledgements

The expert data management of S. Breemans is gratefully acknowledged. We are grateful to Professor Bouillon (Laboratory for Experimental Medicine and Endocrinology) for performing the biochemical measurements. We also wish to thank J. Nijs, H. Borghs, I. Jans and E. Van Herck for skillful technical assistance and A. Vandereijcken for preparing the manuscript. This work was supported in part by a grant from the Sandoz Foundation for Gerontological Research.

Key points

- Ultrasound measurements of the calcaneus continue to decline with ageing, even among the very elderly, presumably reflecting a continuing loss of bone quality.
- Among other factors, deficiency of the growth hormone-IGF-I axis may contribute to this age-related increase in bone fragility.

References


Received in revised form 3 October 1996