1   **Relationships between QUS and HR-pQCT, DXA, and bone turnover markers**

2

3

4   **Ryuji Niimi[1], Ko Chiba[1], Narihiro Okazaki[1], Akihiko Yonekura[1], Masato Tomita[1], Makoto**

5   **Osaki[1]**

6

7   [1]Department of Orthopedic Surgery, Nagasaki University Graduate School of Biomedical Sciences

8

9

10   Corresponding author: Ko Chiba, MD, PhD

11   1-7-1 Sakamoto, Nagasaki 852-8501, Japan,

12   +81-95-819-7321, kohchiba@estate.ocn.ne.jp

13

16

17   Running title: Relationships between QUS, HR-pQCT, DXA, and BTMs

18

19

20

21 **Abstract**

22 **Objectives**

23   Relationship of quantitative ultrasound (QUS) with high-resolution peripheral quantitative

24 computed tomography (HR-pQCT), dual-energy X-ray absorptiometry (DXA), and bone-related

25 biochemical markers was analyzed.

26 **Methods**

27   The subjects were 480 individuals. Speed of sound (SOS) was measured by calcaneal QUS.

28 Volumetric bone mineral density (vBMD) and microarchitecture of trabecular and cortical bone in

29 the distal radius and tibia were assessed by HR-pQCT. Areal bone mineral density (aBMD) in the

30 lumbar spine and proximal femur were measured by DXA. TRACP-5b, P1NP, 25(OH)vitamin D,

31 and pentosidine were evaluated by biochemical tests. The correlation of each parameter was

32 analyzed for all subjects and by sex and age group.

33 **Results**

34   QUS was moderately correlated with Tb.vBMD and Tb.BV/TV in the radius and tibia. No

35 correlation was seen with Ct.vBMD or cortical porosity (Ct.Po). Although a correlation was seen

36 with cortical thickness (Ct.Th) in the tibia in all subjects, no correlation was seen in women aged ≥

37 60 years.

38   QUS showed moderate correlations with aBMD in the proximal femur. Although moderate

39 correlation was seen with aBMD in the lumbar spine in all subjects, no correlation was seen in

40 subjects aged ≥ 60 years. No significant correlations were seen between QUS and biochemical

41 markers.

42 **Conclusions**

43   Moderate correlations were seen between QUS and Tb.vBMD and microarchitecture in the radius

44 and tibia and aBMD of the proximal femur. On the other hand, practically no correlations were seen

45   with Ct.vBMD or Ct.Po and the bone-related biochemical markers. Only in middle age, moderate

46   correlations were seen with Ct.Th in the tibia and with aBMD of the lumbar spine.

47

48

49

50

Introduction

Osteoporotic fractures markedly reduce the activities of daily living of elderly persons and substantially increase healthcare costs. Screening for the early detection of patients with osteoporosis is therefore important to increase the healthy life expectancy of elderly persons and reduce healthcare costs.

To diagnose osteoporosis, measurements of the areal bone mineral density (aBMD) of the lumbar spine and proximal femur by dual-energy X-ray absorptiometry (DXA) are currently used.(1,2) However, DXA requires a large space and is therefore not performed in many clinics. It also involves X-ray exposure, although at a very low dose.

Quantitative ultrasound (QUS) is a method of assessing bone strength using ultrasound mainly in the calcaneus. The device is small and portable, and the measurements can be performed easily and quickly and involve no X-ray exposure. However, QUS is considered to be of limited use in assessing fracture risk, and it is therefore not used to diagnose osteoporosis.

High-resolution peripheral quantitative computed tomography (HR-pQCT) has enabled human bone microarchitecture to be analyzed in vivo, which previously had not been possible.(3) Second-generation HR-pQCT, developed in 2014, has a voxel size of 61 μm, enabling bone microarchitecture to be evaluated more precisely, and it allows the trabecular structure to be measured directly.(4) By analyzing volumetric bone mineral density (vBMD), microarchitecture, and estimated bone strength, HR-pQCT enables fracture risk assessment superior to that allowed by DXA.(5) However, HR-pQCT is expensive, and facilities that can perform it are therefore limited in number. Consequently, it is used mainly for research rather than in routine clinical practice.

To the best of our knowledge, no large studies have examined the relationship between the measurements obtained with calcaneal QUS and the bone microarchitecture analyzed by HR-pQCT. The purpose of this study were to examine the correlations between calcaneal QUS and vBMD and

75   bone microarchitecture of the radius and tibia as analyzed by HR-pQCT, aBMD in the lumbar spine

76   and proximal femur as evaluated by DXA, and bone-related biochemical markers.

77

78

79

80

81 **Methods**

82 **Study participants**

83   The study participants were 480 individuals who participated in a cohort study conducted in

84 Nagasaki City, Japan, that began in December 2015 (Japanese Study of Bone Microstructure and

85 Mineral Density in a Normative Cohort Measured by HR-pQCT: J-CaraT study).

86   The inclusion criteria of the cohort study were age $\geq$ 20 years of either sex, and the exclusion

87 criteria were as follows: history of fragility fractures of the proximal femur or vertebra (excluding

88 occult vertebral fractures); conditions that causes secondary osteoporosis such as use of a

89 corticosteroid, rheumatoid arthritis, paralysis resulting from stroke, severe renal impairment, early

90 menopause, and hyperparathyroidism; and current use of an anti-osteoporosis drug (excluding

91 vitamin D and calcium preparations). The participants were recruited by distributing flyers to local

92 communities (companies, offices in hospitals, public halls, etc.).

93   The participants of the present study consisted of 240 men and 240 women, with 80 men and 80

94 women each in the age ranges of 20 to 39 years, 40 to 59 years, and 60 to 89 years. 80 participants

95 from each age group were selected in the order of registration of the cohort study.

96   This study was approved by the Nagasaki University Hospital Clinical Research Ethics Committee

97 (registration number: 15083105), and informed consent was obtained from all participants before

98 enrollment.

99

100 **QUS**

101   Speed of sound (SOS) (m/s) of right calcaneus was measured using a QUS system (CM-200,

102 FURUNO ELECTRIC CO., LTD., Hyogo, Japan).

103

104 **DXA**

105   aBMD (mg/cm^2) was measured in the lumbar spine and proximal femurs using a DXA system

106   (Lunar Prodigy Advance, GE Lunar, Madison, WI, USA). In the lumbar spine, aBMD was measured

107   at L1 to L4. Vertebrae that were fractured or severely deformed were excluded. For the proximal

108   femurs, aBMD of the total hip and of the femoral neck was measured.

109

110   **Biochemical tests**

111   The following were measured in biochemical tests: serum calcium (Ca), serum phosphorus (P), the

112   bone resorption marker tartrate-resistant acid phosphatase-5b (TRACP-5b), and the bone formation

113   markers total type I procollagen N-terminal propeptide (total P1NP) and 25(OH) vitamin D. In

114   addition, pentosidine was measured as a marker of bone collagen degradation.(6)

115   Serum calcium was corrected if the serum albumin level was < 4 g/dL. The following formula was

116   used for the correction: corrected serum calcium = serum calcium + (4 - serum albumin). TRACP-5b

117   was measured by enzyme immunoassay (EIA) (Osteolinks TRAP-5b, SB Bioscience Co., Ltd.,

118   Tokyo, Japan) and total P1NP by electrochemiluminescence immunoassay (ECLIA) (Elecsys Total

119   P1NP, Roche Diagnostics K.Ks., Tokyo, Japan). Measurement of 25 (OH) vitamin D was performed

120   by chemiluminescent immunoassay (CLIA) (LIAISON 25 OH Vitamin D TOTAL, Hitachi Chemical

121   Diagnostics Systems Co., Ltd., Tokyo, Japan), and pentosidine was measured by high-performance

122   liquid chromatography (HPLC) using a laboratory developed test. The above tests were outsourced

123   to a clinical laboratory testing company (LSI Medience Corporation, Tokyo, Japan).

124

125   **HR-pQCT**

126   The vBMD and bone microarchitecture of the distal radius and tibia were measured on the subject's

127   nondominant side using a second-generation HR-pQCT system (XtreamCT II, Scanco Medical,

128   Brüttisellen, Switzerland). For the radius measurements, scanning was performed over a 10.2-mm

129   range centered on the position at a distance equal to 4% of the forearm length proximal to the ulnar

130   aspect of the surface of the distal radial joint. For the tibia measurements, scanning was performed

131   over a 10.2-mm range centered on the position at a distance equal to 7.3% of the lower leg length

132   proximal to the surface of the distal tibial joint.(7) The following imaging conditions were used

133   based on the standard protocol: tube voltage, 68 kVp; tube current, 1460 µA; integration time, 43

134   ms; number of projections, 900; field of view, 140 mm; matrix, 2304 × 2304; voxel size, 60.7 µm;

135   total number of slices, 168.(7) The scanning time was 2 min, and the effective dose was 5 µSv. All

136   images were evaluated for motion artifacts, and those with artifacts of grade 3 or higher were

137   excluded.(8)

138   The following parameters were measured: total bone mineral density (Tt.BMD, mg/cm^3), total area

139   (Tt.Ar, mm^2), trabecular bone mineral density (Tb.vBMD, mg/cm^3), trabecular bone volume fraction

140   (Tb.BV/TV, %), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), trabecular

141   separation (Tb.Sp, mm), trabecular area (Tb.Ar, mm^2), cortical bone mineral density (Ct.vBMD,

142   mg/cm^3), cortical porosity (Ct.Po, %), cortical thickness (Ct.Th, mm), cortical area (Ct.Ar, mm^2),

143   and cortical perimeter (Ct.Pm, mm).

144

145   **Statistical analysis**

146   The correlations between SOS measured by QUS and the measurement values for DXA, the

147   biochemical tests, and HR-pQCT were determined using Pearson's correlation coefficients. Because

148   SOS was highly correlated with age (age: r = 0.61, height: r = 0.26, weight: r = 0.15), partial

149   correlations adjusted by age was used between SOS and the measurement values for DXA, the

150   biochemical tests, and HR-pQCT. The subjects were divided into 6 groups consisting of a male

151   group and a female group that were each divided into 3 subgroups of subjects aged 20 to 39 years,

152   40 to 59 years, and 60 to 89 years, and the age-adjusted partial correlations were determined in each

153 group. In all of the analyses, a difference was considered significant if the p-value was < 0.01 (IBM

154 SPSS Statistics, version 22.0, IBM Corp, Armonk, NY).

155

156 **Results**

157 **Characteristics of the study participants**

158   The characteristics of the study participants are shown in Table 1. The mean height, weight, and

159 body mass index were 169.3 ± 6.5 cm, 67.8 ± 10.1 kg, 23.6 ± 3.1 kg/m^2 for the men and 156.3 ± 5.4

160 cm, 53.4 ± 9.1 kg, and 21.8 ± 3.5 kg/m^2 for women. The mean Ca, P, TRACP-5b, and total P1NP are

161 within normal range for both men and women, meanwhile the mean 25(OH) vitamin D showed low

162 values with 18.1 ± 6.2 for men and 13.8 ± 5.1 for women.

163

164 **Simple correlations and partial correlations for all participants**

165   The simple correlations and age-adjusted partial correlations between QUS and DXA, the

166 biochemical tests, and HR-pQCT for all subjects are shown in Table 2.

167   SOS was strongly negatively correlated with age for both men and women (overall: r = -0.61, men:

168 r = -0.53, women: r = -0.70). Scatterplots of age and SOS are shown in Fig. 1.

169   SOS showed moderate age-adjusted partial correlations with aBMD of the lumbar spine and the

170 proximal femur measured by DXA (L1 to L4: r = 0.42, total hip: r = 0.49, femoral neck: r = 0.46;

171 Table 2, Fig. 2A).

172   In contrast, no significant correlations were seen between SOS and the biochemical test for Ca, P,

173 TRACP-5b, total P1NP, 25(OH) vitamin D, and pentosidine.

174   In the age-adjusted partial correlations with trabecular bone measured by HR-pQCT, SOS was

175 moderately correlated with Tb.vBMD and Tb.BV/TV in the radius and tibia (r = 0.45, 0.44, 0.52,

176 and 0.52; Table 2, Fig. 2B and 2C). In cortical bone, although moderate correlations with Ct.vBMD

177 in either the radius or tibia were seen in the simple correlation analysis (r = 0.34, 0.48), no

178 correlations were seen in age-adjusted partial analysis. There were weak age-adjusted partial

179 correlations with Ct.Th and Ct.Ar in the tibia (r = 0.39 and r = 0.35), however virtually no

180 correlations were seen with the cortical bone parameters for the radius.,

181

182 **Partial correlations for subgroups by sex and age**

183 The age-adjusted partial correlation between QUS and DXA, the biochemical tests, and HR-pQCT

184 are shown according to sex and age group in Table 3.

185 Moderate correlations were seen between SOS and aBMD of the proximal femur measured by

186 DXA when men and women were examined separately (r = 0.43 to 0.48; Fig. 3A, 3B). Although a

187 weak correlation was seen with aBMD of the lumbar spine in both men and women (r = 0.38, 0.36),

188 no significant correlation was seen for either men or women in the 60 to 89 years age group.

189 No correlations were seen between SOS and the biochemical test parameters when the results were

190 examined by sex and age group.

191 In the correlations with trabecular bone by HR-pQCT, SOS was moderately correlated with

192 Tb.vBMD and Tb.BV/TV in the radius and tibia when men and women were examined separately (r

193 = 0.41 to 0.53; Fig. 3C, 3D, 3E, and 3F). In particular, strong correlations were seen with Tb.vBMD

194 and Tb.BV/TV of the radius in women aged 40 to 59 years (r = 0.64, 0.63).

195 In cortical bone, no correlations were seen for Ct.vBMD or Ct.Po in either the radius or tibia when

196 the results were examined by sex and age group. Although weak correlations were seen with Ct.Th

197 and Ct.Ar of the tibia when men and women were examined separately (r = 0.35 to 0.40), no or weak

198 correlations were seen with Ct.Th and Ct.Ar of the tibia in women aged 60 to 89 years (r = 0.13 to

199 0.38).

200

Discussion

In this study, QUS, DXA, bone-related biochemical markers, and HR-pQCT were assessed in 480 men and women ranging in age from young to elderly, and the correlations between the measurement values were examined. To the best of our knowledge, no large studies have examined the relationships between QUS and HR-pQCT.

The results are summarized in Fig. 4. Although QUS was moderately correlated with aBMD of the lumbar spine in men and women aged 40 to 59 years (r = 0.47, 0.56), no correlation was seen in subjects aged ≥ 60 years. QUS was moderately correlated with aBMD of the proximal femur in men aged 40 to 59 years and in women of all age groups (r = 0.42 to 0.57).

QUS was moderately correlated with Tb.vBMD in the radius and tibia in men aged 40 to 59 years and in women of all age groups (r = 0.42 to 0.64). However, no correlations were seen with Ct.vBMD in the radius and tibia in men and women for any of the age groups. Although moderate correlations were seen with Ct.Th at the tibia in men aged 40 to 59 years and in women aged 20-59 (r = 0.44 to 0.50), no correlations were seen in those aged ≥ 60 years.

1) QUS and DXA

Significant correlation between calcaneal QUS and femoral aBMD in this study (r = 0.31 to 0.57) indicates that calcaneal QUS is useful in screening for decreased aBMD of the femur. Previous studies have also found correlations between QUS and aBMD of the femur (r = 0.35 to 0.72).(9-12) In addition, there have been numerous reports indicating that calcaneal QUS can be used to predict hip fractures.(13-17) The EPIDOS study examined 5662 women aged ≥ 75 years for an average of 2 years and found that the risk ratio for hip fracture was 1.7 to 2.0 for every 1 SD decrease in the QUS measurement value.(15) Krieg et al. reported that, in postmenopausal women and men aged ≥ 65 years, QUS predicted fragility hip fractures and non-vertebral fractures independently of aBMD

225   measured by DXA.(16)

226   On the other hand, no correlation between calcaneal QUS and aBMD of the lumbar spine was seen

227   in subjects aged ≥ 60 years. This might be attributed to the effect of spinal osteoarthritis and aortic

228   calcification on aBMD of the lumbar spine in elderly subjects. Significant correlation only in men

229   and women aged 40 to 59 years (r = 0.47, 0.56) suggests that calcaneal QUS is useful for early

230   screening of decreased aBMD of the lumbar spine in individuals in middle age.

231

232   **2) QUS and biochemical tests**

233   No correlations were seen between calcaneal QUS and bone turnover markers (TRACP-5b and

234   P1NP) in this study. Predicting the status of bone turnover is difficult with calcaneal QUS. There is a

235   5-year longitudinal study in women aged ≥ 75 years examining the correlations between QUS and

236   the annual percent change in bone turnover markers. The correlation coefficients were -0.10 for both

237   TRACP-5b and serum cross-linked C-telopeptide of type I collagen (CTX) and -0.16 for

238   osteocalcin.(18)

239   In the present study, no correlation was seen between calcaneal QUS and 25 (OH) vitamin D or

240   pentosidine. In a cross-sectional study investigating the relationship between calcaneal QUS and 25

241   (OH) vitamin D conducted by Kauppi et al.,(19) the multiple regression coefficients obtained in a

242   multiple regression analysis adjusted for factors such as height, weight, menopause status, and

243   lifestyle were 0.18 for SOS and 0.07 for broadband ultrasound attenuation (BUA) in men and 0.11

244   for SOS and 0.07 for BUA in women.

245   To the best of our knowledge, there have been no previous studies investigating the relationship

246   between calcaneal QUS and pentosidine.

247

248   **3) QUS and HR-pQCT**

249   Significant correlations between calcaneal QUS and Tb.vBMD in the radius and tibia (r = 0.32 to

250   0.64) indicate that QUS is useful for screening of deterioration of the trabecular bone

251   microarchitecture in the extremities (Fig. 5). Many previous studies have reported postmenopausal

252   women with a history of fragility fracture had low values of Tb.vBMD measured by HR-pQCT,[20-

253   24] indicating that QUS has a potential ability to indirectly predict fragility fracture.

254   On the other hand, no correlations between calcaneal QUS and Ct.vBMD or Ct.Po in the radius and

255   tibia indicate that current calcaneal QUS cannot predict deterioration in the cortical bone. Because

256   the calcaneus consists largely of trabecular bone, calcaneal QUS may not truly reflect the status of

257   cortical bone. In recent years, the axial transmission technique, which selectively evaluates cortical

258   bone by ultrasound, has been investigated.[25-28]

259   In the present study, calcaneal QUS was moderately correlated with Ct.Th of the tibia only in men

260   and women aged 40 to 59 years (r = 0.49, 0.50). Calcaneal QUS may therefore be useful for early

261   screening of decreased Ct.Th at the tibia in individuals in middle age. A previous study of HR-pQCT

262   found that Ct.Th at the tibia was 15% lower in postmenopausal women with a history of bone

263   fracture as compared with women with no such history.[21]

264

265   **Limitations**

266   There are several limitations in the present study. First, this study recruited participants by

267   distributing flyers to local communities. Therefore, self-selection bias and healthy worker bias were

268   inevitable. Second, measurement values of calcaneal QUS can be affected by the temperature, size,

269   and edema of the heel.[29-31] Measurement values of vitamin D are affected by season and diet. The

270   data in the present study were collected from young and elderly individuals of varying body types

271   without specifying the season or time when the examinations were performed. Consequently, these

272   factors may have contributed to variability in the measurement values.

273

274 **Conclusions**

275   Calcaneal QUS showed moderate correlations with trabecular vBMD and bone microarchitecture in

276 the radius and tibia measured by HR-pQCT and with aBMD of the proximal femur measured by

277 DXA. However, QUS showed no correlations with cortical vBMD or porosity in the radius and tibia

278 and with bone turnover markers, 25 (OH) vitamin D, or pentosidine. QUS showed moderate

279 correlations with cortical thickness in the tibia and with aBMD of the lumbar spine only in subjects

280 aged 40 to 59 years.

281

282

283

284

285

299

300

301

302

303

304

305

306   **References**

307   (1) Kanis, J. A. (2002). Diagnosis of osteoporosis and assessment of fracture risk. The Lancet,

308   359(9321), 1929–1936.

309   (2) Soen, S., Fukunaga, M., Sugimoto, T., Sone, T., Fujiwara, S., Endo, N., Gorai, I., Shiraki, M.,

310   Hagino, H., Hosoi, T., Ohta, H., Yoneda, T., & Tomomitsu, T. (2013). Diagnostic criteria for

311   primary osteoporosis: year 2012 revision. Journal of Bone and Mineral Metabolism, 31(3), 247–

312   257.

313   (3) Burghardt, A. J., Link, T. M., & Majumdar, S. (2011). High-resolution Computed Tomography

314   for Clinical Imaging of Bone Microarchitecture. Clinical Orthopaedics & Related Research,

315   469(8), 2179–2193.

316   (4) Manske, S. L., Zhu, Y., Sandino, C., & Boyd, S. K. (2015). Human trabecular bone

317   microarchitecture can be assessed independently of density with second generation HR-pQCT.

318   Bone, 79, 213–221.

319   (5) Samelson, E. J., Broe, K. E., Xu, H., Yang, L., Boyd, S., Biver, E., Szulc, P., Adachi, J., Amin,

320   S., Atkinson, E., Berger, C., Burt, L., Chapurlat, R., Chevalley, T., Ferrari, S., Goltzman, D.,

321   Hanley, D. A., Hannan, M. T., Khosla, S., … Bouxsein, M. L. (2019). Cortical and trabecular

322   bone microarchitecture as an independent predictor of incident fracture risk in older women and

323   men in the Bone Microarchitecture International Consortium (BoMIC): a prospective study. The

324   Lancet Diabetes & Endocrinology, 7(1), 34–43.

325   (6) Saito, M., & Marumo, K. (2010). Collagen cross-links as a determinant of bone quality: a

326   possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus.

327   Osteoporosis International, 21(2), 195–214.

328   (7) Whittier, D. E., Boyd, S. K., Burghardt, A. J., Paccou, J., Ghasem-Zadeh, A., Chapurlat, R.,

329   Engelke, K., & Bouxsein, M. L. (2020). Guidelines for the assessment of bone density and

330 microarchitecture in vivo using high-resolution peripheral quantitative computed tomography.

331 Osteoporosis International, 31(9), 1607–1627.

332 (8) Pialat, J., Burghardt, A. J., Sode, M., Link, T. M., & Majumdar, S. (2012). Visual grading of

333 motion induced image degradation in high resolution peripheral computed tomography: Impact

334 of image quality on measures of bone density and micro-architecture. Bone, 50(1), 111–118.

335 (9) Herd, R. J. M., Blake, G. M., Miller, C. G., Parker, J. C., & Fogelman, I. (1994). The ultrasonic

336 assessment of osteopenia as defined by dual X-ray absorptiometry. The British Journal of

337 Radiology, 67(799), 631–635.

338 (10) Lees, B., & Stevenson, J. C. (1993). Preliminary evaluation of a new ultrasound bone

339 densitometer. Calcified Tissue International, 53(3), 149–152.

340 (11) Rosenthall, L., Tenenhouse, A., & Caminis, J. (1995). A correlative study of ultrasound

341 calcaneal and dual-energy X-ray absorptiometry bone measurements of the lumbar spine and

342 femur in 1000 women. European Journal of Nuclear Medicine, 22(5), 402–406.

343 (12) Schott, A. M., Hans, D., Sornay-Rendu, E., Delmas, P. D., & Meunier, P. J. (1993). Ultrasound

344 measurements on os calcis: Precision and age-related changes in a normal female population.

345 Osteoporosis International, 3(5), 249–254.

346 (13) Bauer, D. C., Glüer, C. C., Cauley, J. A., Vogt, T. M., Ensrud, K. E., Genant, H. K., & Black, D.

347 M. (1997). Broadband ultrasound attenuation predicts fractures strongly and independently of

348 densitometry in older women. A prospective study. Study of Osteoporotic Fractures Research

349 Group. Archives of Internal Medicine, 157(6), 629–634.

350 (14) Fujiwara, S., Sone, T., Yamazaki, K., Yoshimura, N., Nakatsuka, K., Masunari, N., Fujita, S.,

351 Kushida, K., & Fukunaga, M. (2005). Heel bone ultrasound predicts non-spine fracture in

352 Japanese men and women. Osteoporosis International, 16(12), 2107–2112.

353 (15) Hans, D., Dargent-Molina, P., Schott, A., Sebert, J., Cormier, C., Kotzki, P., Delmas, P.,

354   Pouilles, J., Breart, G., & Meunier, P. (1996). Ultrasonographic heel measurements to predict

355   hip fracture in elderly women: the EPIDOS prospective study. The Lancet, 348(9026), 511–514.

356   (16) Krieg, M.-A., Barkmann, R., Gonnelli, S., Stewart, A., Bauer, D. C., Del Rio Barquero, L.,

357   Kaufman, J. J., Lorenc, R., Miller, P. D., Olszynski, W. P., Poiana, C., Schott, A.-M., Lewiecki,

358   E. M., & Hans, D. (2008). Quantitative Ultrasound in the Management of Osteoporosis: The

359   2007 ISCD Official Positions. Journal of Clinical Densitometry, 11(1), 163–187.

360   (17) Marín, F., González-Macías, J., Díez-Pérez, A., Palma, S., & Delgado-Rodríguez, M. (2006).

361   Relationship Between Bone Quantitative Ultrasound and Fractures: A Meta-Analysis. Journal of

362   Bone and Mineral Research, 21(7), 1126–1135.

363   (18) Lenora, J., Gerdhem, P., Obrant, K. J., & Ivaska, K. K. (2009). Bone turnover markers are

364   correlated with quantitative ultrasound of the calcaneus: 5-year longitudinal data. Osteoporosis

365   International, 20(7), 1225–1232.

366   (19) Kauppi, M., Impivaara, O., Mäki, J., Heliövaara, M., Marniemi, J., Montonen, J., & Jula, A.

367   (2009). Vitamin D status and common risk factors for bone fragility as determinants of

368   quantitative ultrasound variables in a nationally representative population sample. Bone, 45(1),

369   119–124.

370   (20) Cohen, A., Liu, X. S., Stein, E. M., McMahon, D. J., Rogers, H. F., LeMaster, J., Recker, R. R.,

371   Lappe, J. M., Guo, X. E., & Shane, E. (2009). Bone Microarchitecture and Stiffness in

372   Premenopausal Women with Idiopathic Osteoporosis. The Journal of Clinical Endocrinology &

373   Metabolism, 94(11), 4351–4360.

374   (21) Stein, E. M., Liu, X. S., Nickolas, T. L., Cohen, A., Thomas, V., McMahon, D. J., Zhang, C.,

375   Yin, P. T., Cosman, F., Nieves, J., Guo, X. E., & Shane, E. (2010). Abnormal microarchitecture

376   and reduced stiffness at the radius and tibia in postmenopausal women with fractures. Journal of

377   Bone and Mineral Research, 25(12), 2572–2581.

378   (22) Sornay-Rendu, E., Boutroy, S., Duboeuf, F., & Chapurlat, R. D. (2017). Bone Microarchitecture

379   Assessed by HR-pQCT as Predictor of Fracture Risk in Postmenopausal Women: The OFELY

380   Study. Journal of Bone and Mineral Research, 32(6), 1243–1251.

381   (23) Vico, L., Zouch, M., Amirouche, A., Frère, D., Laroche, N., Koller, B., Laib, A., Thomas, T., &

382   Alexandre, C. (2008). High-Resolution pQCT Analysis at the Distal Radius and Tibia

383   Discriminates Patients With Recent Wrist and Femoral Neck Fractures. Journal of Bone and

384   Mineral Research, 23(11), 1741–1750.

385   (24) Liu, X. S., Stein, E. M., Zhou, B., Zhang, C. A., Nickolas, T. L., Cohen, A., Thomas, V.,

386   McMahon, D. J., Cosman, F., Nieves, J., Shane, E., & Guo, X. E. (2012). Individual trabecula

387   segmentation (ITS)-based morphological analyses and microfinite element analysis of HR-

388   pQCT images discriminate postmenopausal fragility fractures independent of DXA

389   measurements. Journal of Bone and Mineral Research, 27(2), 263–272.

390   (25) Chiba, K., Suetoshi, R., Cretin, D., Arai, T., Kawajiri, T., Okayama, A., Tsuji, S., Okazaki, N.,

391   Osaki, M., & Yoh, K. (2021). Development of a QUS Device to Evaluate Deterioration of

392   Cortical Bone: Verification by HR-pQCT and Measurements in Healthy Individuals and

393   Dialysis Patients. Journal of Clinical Densitometry, 24(1), 94–105.

394   (26) Muller, M., Moilanen, P., Bossy, E., Nicholson, P., Kilappa, V., Timonen, J., Talmant, M.,

395   Cheng, S., & Laugier, P. (2005). Comparison of three ultrasonic axial transmission methods for

396   bone assessment. Ultrasound in Medicine & Biology, 31(5), 633–642.

397   (27) Schneider, J., Ramiandrisoa, D., Armbrecht, G., Ritter, Z., Felsenberg, D., Raum, K., &

398   Minonzio, J.-G. (2019). In Vivo Measurements of Cortical Thickness and Porosity at the

399   Proximal Third of the Tibia Using Guided Waves: Comparison with Site-Matched Peripheral

400   Quantitative Computed Tomography and Distal High-Resolution Peripheral Quantitative

401   Computed Tomography. Ultrasound in Medicine & Biology, 45(5), 1234–1242.

402   (28) Tatarinov, A., Egorov, V., Sarvazyan, N., & Sarvazyan, A. (2014). Multi-frequency axial

403   transmission bone ultrasonometer. Ultrasonics, 54(5), 1162–1169.

404   (29) Chappard, C., Berger, G., Roux, C., & Laugier, P. (1999). Ultrasound Measurement on the

405   Calcaneus: Influence of Immersion Time and Rotation of the Foot. Osteoporosis International,

406   9(4), 318–326.

407   (30) Iki, M., Kajita, E., Mitamura, S., Nishino, H., Yamagami, T., & Nagahama, N. (1999). Precision

408   of Quantitative Ultrasound Measurement of the Heel Bone and Effects of Ambient Temperature

409   on the Parameters. Osteoporosis International, 10(6), 462–467.

410   (31) Paggiosi, M. A., Blumsohn, A., Barkmann, R., & Eastell, R. (2005). Effect of Temperature on

411   the Longitudinal Variability of Quantitative Ultrasound Variables. Journal of Clinical

412   Densitometry, 8(4), 436–444.

413

414

415

416   **Figure legends**

417

418   **Fig.1**   Correlations between age and SOS in all participants (A), men (B), and women (C).

419

420   **Fig.2**   Correlations between SOS, aBMD of the total hip (A), and Tb.vBMD at the radius (B) and

421   tibia (C).

422

423   **Fig.3**   Correlations between SOS, aBMD of the total hip (A and B), and Tb.vBMD at the radius

424   (C and D) and tibia (E and F) in men and women.

425

426   **Fig.4**   Relationship of calcaneal QUS to DXA (lumbar spine and total hip) and HR-pQCT (distal

427   radius and tibia). Partial correlation coefficients are shown for three age groups (20-39, 40-59, 60-89

428   years) in men and women. Bold indicates r>0.4.

429

430   **Fig.5**   Representative HR-pQCT images of distal tibia of women with higher SOS (A) and with

431   lower SOS (B).

432

433   **Table 1**   Characteristics of study participants

434

435   **Table 2**   Pearson's correlation coefficients and partial correlation coefficients adjusted for age

436   between SOS and other measurements.

437   * p<0.01, ** p<0.001, r >0.4 and r >0.6 are highlighted in yellow and red, respectively

438

439   **Table 3**   Partial correlation coefficients adjusted for age between SOS and other measurements

440   among each generation in men and women

441   * p<0.01, ** p<0.001, r >0.4 and r >0.6 are highlighted in yellow and red, respectively

442

Fig.1. Correlations between age and SOS in all participants (A), men (B), and women (C).
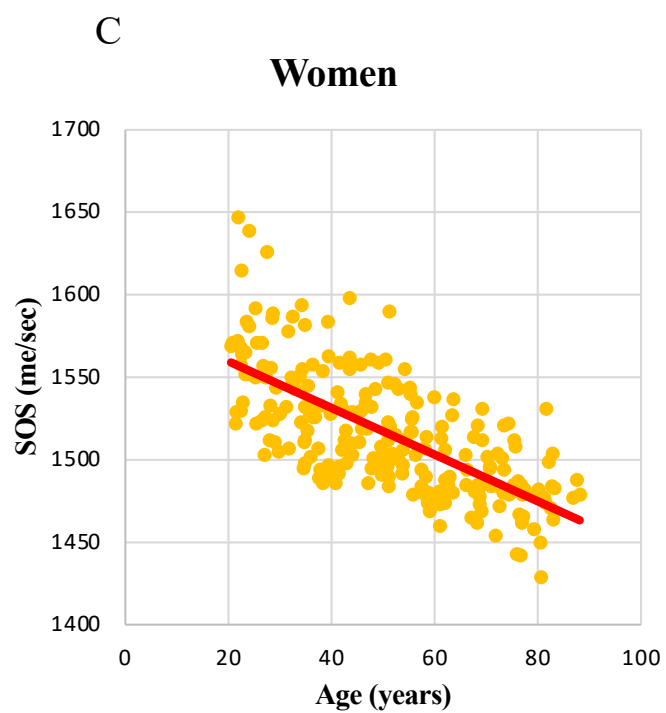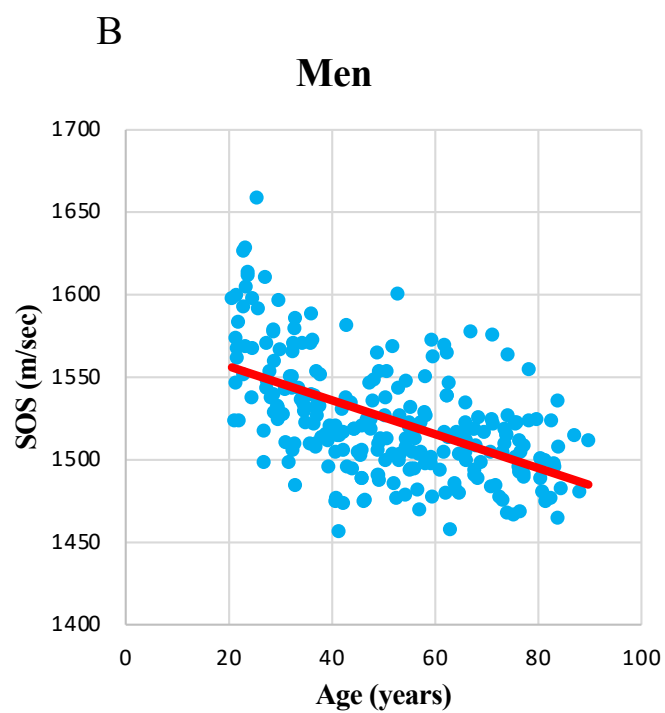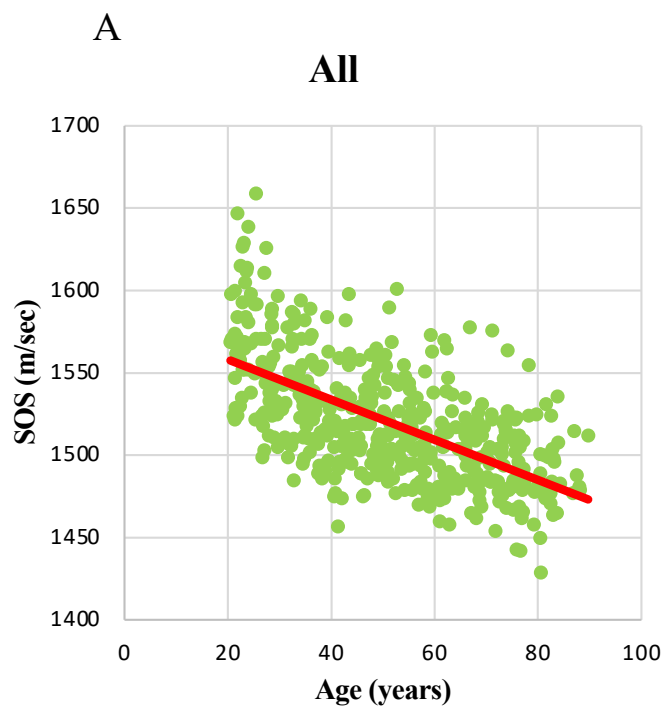
Fig.2. Correlations between SOS, aBMD of the total hip (A), and Tb.vBMD at the radius (B) and tibia (C).
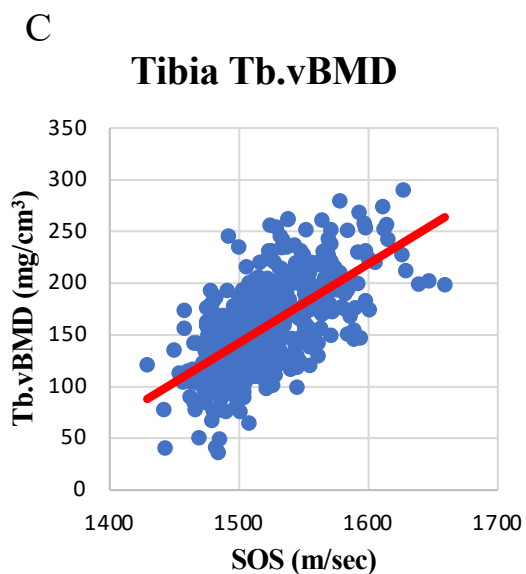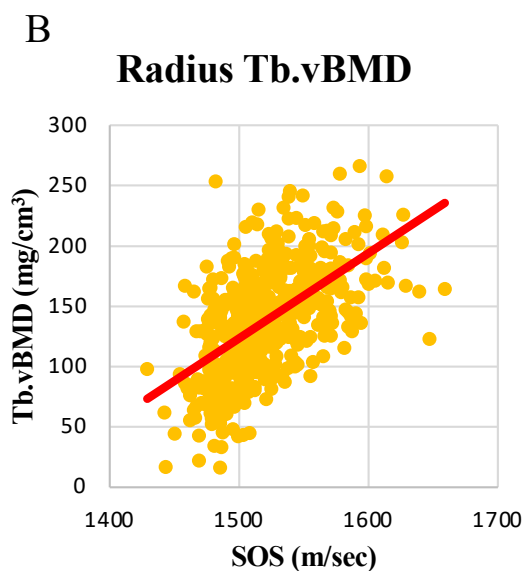
A

B

C
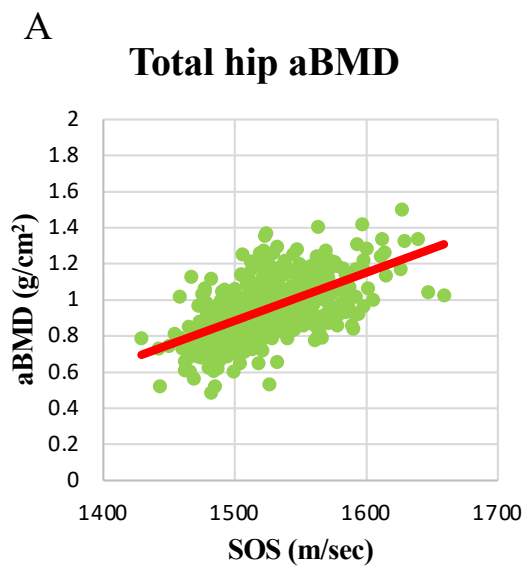
Fig.3. Correlations between SOS, aBMD of the total hip (A and B), and Tb.vBMD at the radius (C and D) and tibia (E and F) in men and women.
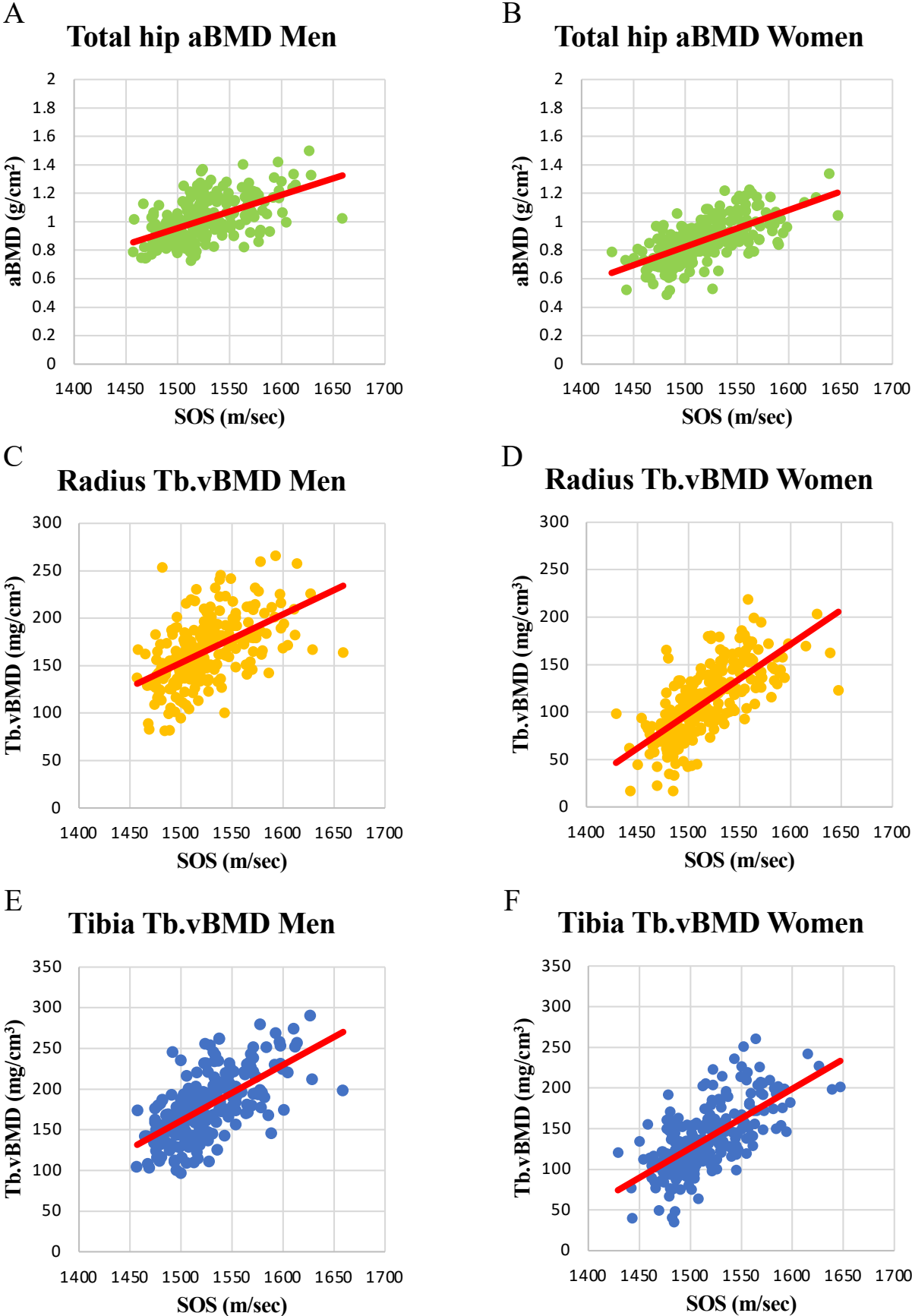
Fig.4 Relationship of calcaneal QUS to DXA (lumbar spine and total hip) and HR-pQCT (distal radius and tibia). Partial correlation coefficients are shown for three age groups (20-39, 40-59, 60-89 years) in men and women. Bold indicates r>0.4.

Correlations with DXA at **lumbar spine**.
years (20-39, 40-59, 60-89)
aBMD: Men   r= 0.32, **0.47**, 0.17
Women r= 0.38, **0.56**, 0.15

Correlations with HR-pQCT at **distal radius**.
Tb.vBMD: Men   r= 0.32, **0.58**, 0.39
Women   r= **0.44, 0.64, 0.46**
Ct.vBMD : Men   r= 0.22, 0.20, 0.07
Women   r= 0.04, 0.22, 0.11
Ct.Th :   Men   r= 0.15, 0.24, 0.04
Women   r= 0.13, 0.30, 0.23

Correlations with DXA at **total hip**.
aBMD: Men   r= 0.36, **0.57**, 0.31
Women r= **0.48, 0.45, 0.42**

Correlations with HR-pQCT at **distal tibia**.
Tb.vBMD: Men   r= 0.33, **0.51, 0.42**
Women   r= **0.52, 0.56, 0.45**
Ct.vBMD : Men   r= 0.08, 0.33, 0.13
Women   r= 0.16, 0.35, -0.08
Ct.Th :   Men   r= 0.31, **0.49**, 0.38
Women   r= **0.44, 0.50**, 0.21

Fig.5. Representative HR-pQCT images of distal tibia of women with higher SOS (A) and with lower SOS (B).

Table 1 Characteristics of study participants

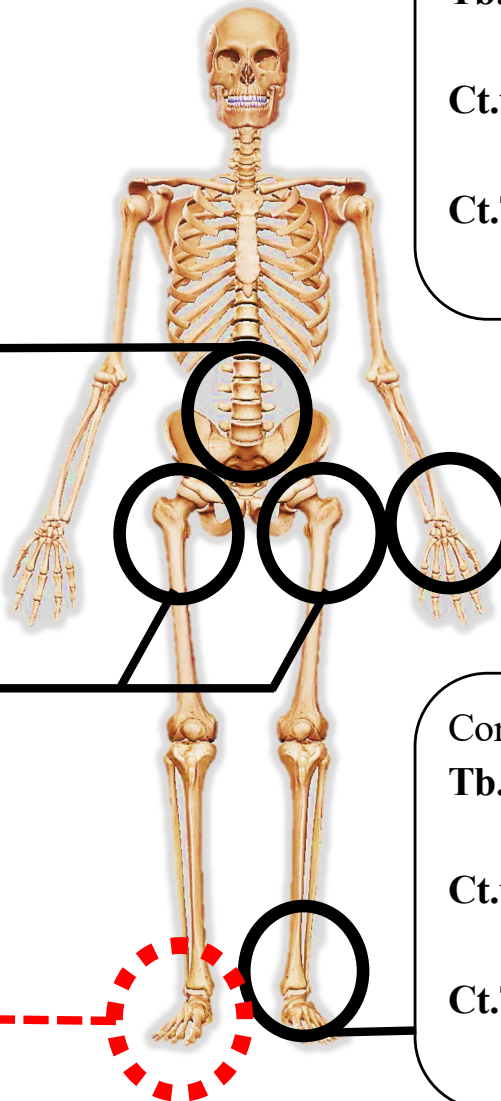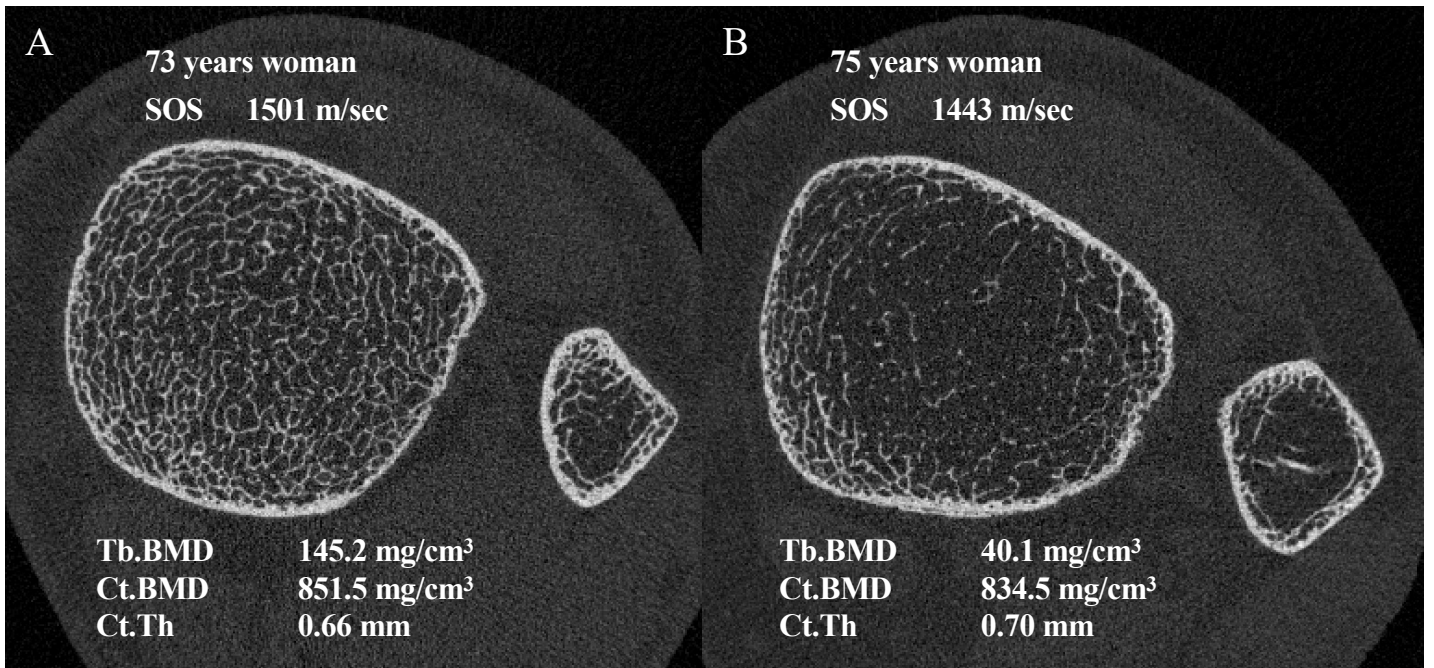|	All (N= 480)	Men (N= 240)	Women (N= 240)
Age (years)	50.8±18.4	50.9±18.5	50.7±18.4
Age range (years)	20.5 - 89.7	20.6 - 89.7	20.5 - 88.1
20-39	160	80	80
40-59	160	80	80
60+	160	80	80
Height (cm)	162.78±8.8	169.26±6.5	156.30±5.4
Weight (kg)	60.57±12.0	67.80±10.1	53.35±9.1
BMI (kg/m²)	22.74±3.4	23.64±3.1	21.83±3.5
QUS			
SOS (m/sec)	1520.53±36.6	1524.85±35.7	1516.20±37.1
DXA			
Lumbar spine aBMD (g/cm^2)	1.10±0.2	1.16±0.2	1.03±0.2
Total hip aBMD (g/cm^2)	0.94±0.2	1.01±0.1	0.87±0.1
Femoral neck aBMD (g/cm^2)	0.88±0.2	0.95±0.2	0.82±0.1
Laboratory			
Ca (m/L)	9.34±0.4	9.37±0.4	9.31±0.3
P (m/L)	3.37±0.5	3.24±0.5	3.51±0.5
TRACP-5b (mU/dL)	357.07±148.6	339.63±122.9	374.52±169.0
total P1NP (µg/L)	52.32±21.5	49.85±21.3	54.79±21.5
25(OH)D (ng/mL)	15.92±6.1	18.07±6.2	13.77±5.1
Pentosidine (pmol/mL)	25.33±8.5	24.87±8.2	25.79±8.8
HR-pQCT			
Radius			
Tt.vBMD (mg/cm³)	275.81±71.1	310.10±57.0	241.53±67.3
Tt.Ar (mm^2)	310.15±54.5	345.37±46.3	274.94±36.4
Tb.vBMD (mg/cm³)	137.92±45.9	165.69±34.6	110.16±38.4
Tb.BV/TV (%)	0.20±0.1	0.24±0.0	0.17±0.0
Tb.N (/mm)	1.28±0.2	1.37±0.2	1.19±0.2
Tb.Th (µm)	0.23±0.0	0.24±0.0	0.21±0.0
Tb.Sp (µm)	0.77±0.2	0.68±0.1	0.86±0.3
Tb.Ar (mm^2)	255.24±49.7	279.65±47.5	230.83±38.6
Ct.vBMD (mg/cm³)	880.30±68.9	884.75±53.5	875.85±81.4
Ct.Po (%)	0.74±0.6	1.04±0.7	0.45±0.3
Ct.Th (mm)	0.95±0.2	1.07±0.2	0.82±0.2
Ct.Ar (mm^2)	58.88±15.2	69.94±11.3	47.82±9.4
Ct.Pm (mm)	73.65±7.1	78.39±5.7	68.92±4.9
Tibia			
Tt.vBMD (mg/cm³)	282.42±68.0	312.89±56.0	251.95±65.4
Tt.Ar (mm^2)	729.97±110.5	796.94±93.9	663.01±81.4
Tb.vBMD (mg/cm³)	158.03±44.7	178.10±39.1	137.95±40.9
Tb.BV/TV (%)	0.24±0.1	0.26±0.1	0.21±0.1
Tb.N (/mm)	1.25±0.2	1.29±0.2	1.22±0.2
Tb.Th (µm)	0.25±0.0	0.26±0.0	0.24±0.0
Tb.Sp (µm)	0.78±0.2	0.75±0.1	0.81±0.2
Tb.Ar (mm^2)	608.91±101.0	650.82±96.3	567.01±87.4
Ct.vBMD (mg/cm³)	888.04±68.5	890.61±54.1	885.47±80.4
Ct.Po (%)	2.77±1.5	3.13±0.0	2.40±1.4
Ct.Th (mm)	1.41±0.4	1.63±0.3	1.19±0.3
Ct.Ar (mm^2)	126.51±34.4	151.83±24.7	101.19±21.8
Ct.Pm (mm)	105.50±8.4	110.66±6.9	100.33±6.2

Table 2 Pearson's correlation coefficients and partial correlation coefficients adjusted for age between SOS and other measurements.

		All (N=480)	
		Correlation	Partial correlation adjusted for age
Age		-0.61[**]	
Height		0.26[**]	
Weight		0.15[**]	
BMI		0.02	
DXA			
	Lumbar spine aBMD	0.41[**]	0.42[**]
	Total hip aBMD	0.60[**]	0.49[**]
	Femoral neck aBMD	0.62[**]	0.46[**]
Laboratory			
	Ca	0.10	-0.02
	P	-0.07	-0.11
	TRACP-5b	-0.25[**]	-0.07
	total P1NP	0.07	-0.01
	25(OH)D	-0.05	0.10
	Pentosidine	-0.27[**]	0.07
HR-pQCT			
Radius	Tt.vBMD	0.49[**]	0.33[**]
	Tt.Ar	-0.08	0.05
	Tb.vBMD	0.56[**]	0.45[**]
	Tb.BV/TV	0.55[**]	0.44[**]
	Tb.N	0.56[**]	0.39[**]
	Tb.Th	0.21[**]	0.25[**]
	Tb.Sp	-0.47[**]	-0.30[**]
	Tb.Ar	-0.15[**]	0.00
	Ct.vBMD	0.34[**]	0.07
	Ct.Po	-0.18[**]	0.17[**]
	Ct.Th	0.28[**]	0.20[**]
	Ct.Ar	0.21[**]	0.19[**]
	Ct.Pm	-0.14[*]	0.05
Tibia	Tt.vBMD	0.66[**]	0.51[**]
	Tt.Ar	-0.05	0.02
	Tb.vBMD	0.63[**]	0.52[**]
	Tb.BV/TV	0.62[**]	0.52[**]
	Tb.N	0.51[**]	0.33[**]
	Tb.Th	0.36[**]	0.35[**]
	Tb.Sp	-0.49[**]	-0.30[**]
	Tb.Ar	-0.21[**]	-0.10
	Ct.vBMD	0.48[**]	0.11
	Ct.Po	-0.24[**]	0.10
	Ct.Th	0.51[**]	0.39[**]
	Ct.Ar	0.45[**]	0.35[**]
	Ct.Pm	-0.08	0.03

* p<0.01, ** p<0.001, r >0.4 and r >0.6 are highlighted in yellow and red respectively

Table 3 Partial correlation coefficients adjusted for age between SOS and other measurements among each generation in men and women

		Men All N= 240	Men 20-39 N= 80	Men 40-59 N= 80	Men 60-89 N= 80	Women All N= 240	Women 20-39 N= 80	Women 40-59 N= 80	Women 60-88 N= 80
DXA									
	Lumbar spine aBMD	0.38**	0.32*	0.47**	0.17	0.36**	0.38**	0.56**	0.15
	Total hip aBMD	0.48**	0.36*	0.57**	0.31*	0.47**	0.48**	0.45**	0.42**
	Femoral neck aBMD	0.44**	0.33	0.51**	0.29	0.43**	0.38	0.45**	0.41**
Laboratory									
	Ca	-0.05	-0.20	-0.11	0.13	0.02	0.09	-0.15	0.15
	P	-0.09	-0.28	-0.13	0.06	0.00	0.11	-0.07	-0.04
	TRACP-5b	0.05	-0.06	-0.10	0.00	-0.08	-0.27	-0.08	-0.04
	total P1NP	0.11	-0.02	-0.23	-0.12	-0.01	-0.03	-0.15	-0.02
	25(OH)D	0.12	0.16	-0.08	0.06	-0.05	-0.07	-0.20	0.07
	Pentosidine	0.13	0.10	0.04	0.16	0.04	0.05	0.01	-0.04
HR-pQCT									
Radius	Tt.vBMD	0.22**	0.28	0.40**	0.26	0.35**	0.28	0.47**	0.39**
	Tt.Ar	-0.07	-0.04	-0.04	-0.26	-0.10	-0.06	-0.09	-0.10
	Tb.vBMD	0.41**	0.32*	0.58**	0.39**	0.51**	0.44**	0.64**	0.46**
	Tb.BV/TV	0.41**	0.30*	0.57**	0.38**	0.51**	0.43**	0.63**	0.44**
	Tb.N	0.33**	0.35*	0.41**	0.40**	0.38**	0.36*	0.54**	0.31*
	Tb.Th	0.16	0.01	0.44**	0.05	0.26**	0.19	0.40**	0.17
	Tb.Sp	-0.37**	-0.39**	-0.45**	-0.43**	-0.23**	-0.33*	-0.48**	-0.17
	Tb.Ar	-0.08	-0.08	-0.10	-0.24	-0.14	-0.09	-0.17	-0.13
	Ct.vBMD	-0.02	0.22	0.20	0.07	0.07	0.04	0.22	0.11
	Ct.Po	0.04	-0.01	0.11	-0.08	0.17	0.33*	0.07	0.20
	Ct.Th	0.05	0.15	0.24	0.04	0.18*	0.13	0.30*	0.23
	Ct.Ar	0.04	0.17	0.26	-0.06	0.17	0.12	0.33*	0.20
	Ct.Pm	-0.10	-0.10	-0.06	-0.24	-0.08	-0.10	-0.02	-0.06
Tibia	Tt.vBMD	0.48**	0.34*	0.55**	0.46**	0.53**	0.51**	0.61**	0.38**
	Tt.Ar	-0.03	0.02	0.08	-0.28	-0.19*	-0.19	-0.19	-0.18
	Tb.vBMD	0.51**	0.33*	0.51**	0.42**	0.53**	0.52**	0.56**	0.45**
	Tb.BV/TV	0.52**	0.34*	0.51**	0.41**	0.53**	0.51**	0.56**	0.46**
	Tb.N	0.29**	0.34*	0.27	0.21	0.32**	0.42**	0.35**	0.25
	Tb.Th	0.32**	0.07	0.35*	0.25	0.37**	0.33**	0.39**	0.26
	Tb.Sp	-0.32**	-0.35*	-0.33	-0.24	-0.23**	-0.44**	-0.39*	-0.16
	Tb.Ar	-0.12	-0.08	-0.03	-0.35*	-0.26**	-0.27	-0.29	-0.19
	Ct.vBMD	0.04	0.08	0.33*	0.13	0.10	0.16	0.35*	-0.08
	Ct.Po	0.08	-0.02	-0.02	0.03	0.07	0.14	-0.20	0.22
	Ct.Th	0.38**	0.31*	0.49**	0.38**	0.40**	0.44**	0.50**	0.21
	Ct.Ar	0.37**	0.35*	0.54**	0.28	0.35**	0.39	0.46**	0.13
	Ct.Pm	-0.02	0.05	0.06	-0.26	-0.18*	-0.17	-0.16	-0.18

* p<0.01, ** p<0.001, r >0.4 and r >0.6 are highlighted in yellow and red respectively