ANTICANCER RESEARCH *38*: 6465-6471 (2018)
doi:10.21873/anticanres.13009

Impact of Osteopenia in Liver Cirrhosis: Special Reference to Standard Bone Mineral Density with Age

TAKEO TOSHIMA, TOMOHARU YOSHIZUMI, TORU IKEGAMI, NOBORU HARADA, SHINJI ITOH, YOHEI MANO, TAKASHI MOTOMURA, YUJI SOEJIMA and YOSHIHIKO MAEHARA

Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Abstract. *Background/Aim: Computed tomography (CT) has recently been applied to measure bone mineral density (BMD). However, the definition of osteopenia, which means depletion of BMD, using CT remains controversial. The aim of this study was to establish formulae to calculate standard BMD. Patients and Methods: Fifty healthy donors for liver transplantation underwent CT. BMD was measured as cross-sectional average pixel density (Hounsfield units) only in trabecular-bone at Th11 bottom. Validation was performed on another 50 healthy donors to check the accuracy of formulae for standard BMD. Results: Measured BMD was significantly correlated with age in both males and females ($p<0.0001$). The formulae to calculate standard BMD were 308.82-2.49 × Age in males and 311.84-2.41 × Age in females. Estimated BMD was significantly correlated with measured BMD in males and females ($p<0.0001$). Conclusion: Osteopenia can be defined by the difference between measured data and calculated data using our new formula based on each age.*

Osteopenia, defined as abnormally low bone mineral density (BMD), is a common musculoskeletal disorder in the elderly. It is considered to be caused by a combination of physical, metabolic, and endocrine factors (1-3). In general, a decreased physical activity may reduce the mechanical loading to both bone and skeletal muscle, and result in a decrease of BMD as osteopenia (4). Depletion of BMD can predict survival in patients with various kinds of cancers (5-8) or patients with liver cirrhosis (9). Like sarcopenia, the loss of skeletal muscle, osteopenia is a common condition in patients with liver cirrhosis.

Recently, Sharma *et al*. (9) demonstrated that the low levels of BMD were independently associated with post-liver transplantation (LT) mortality in patients with liver cirrhosis with hepatocellular carcinoma (HCC). They analyzed BMD by measuring the average pixel density of trabecular bone at the bottom of the eleventh thoracic vertebral level (Th11) on preoperative enhanced computed tomography (CT). In their report, the median BMD was 185 Hounsfield units (HU) and the 25th and 75th percentile were 162 and 216 HU, respectively. To further explore the association between BMD and post-LT mortality, they actually applied the cutoff of BMD <160 HU in accordance with a previous study (10), in a single uniform way and independently of age and sex. However, the authors did not use BMD to determine osteopenia in their study. In patients with severe liver disease as cirrhosis or advanced HCC, deceased metabolism of calcium or the change in the synthesis of steroid hormone may also affect the bone metabolism, and in many cases these changes are exacerbated by aging (11, 12). Thus, the definition of osteopenia using BMD has been set using an unfounded cut-off level by CT measurement, and has not been accurately determined as yet.

In this study, we retrospectively studied healthy Japanese adult living donors for liver transplantation to establish formulae to calculate the standard BMD, to enable an easy and accurate definition of osteopenia.

Patients and Methods

Control subjects. Data on 50 healthy adults (25 males and 25 females, aged 20-64 years) who hoped to be liver donors were reviewed. Donor candidates who had compressed spine fracture or other bone diseases were excluded from this study, because these conditions affected BMD. All 50 candidates were eligible to be donors and underwent left or right hepatectomy after several studies, including 2-mm-slice abdominal CT. These CT images were used for determining the quantity of BMD and osteopenia.

Correspondence to: Takeo Toshima, MD, Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel: +81 926425466, Fax: +81 926425482, e-mail: toshima@surg2.med.kyushu-u.ac.jp

Key Words: Osteopenia, bone mineral density, sarcopenia, liver transplantation, liver cirrhosis.

Bodyweight (BW) and height recorded on the donor charts were used for calculating body surface area (BSA) and body mass index (BMI). Equations for BSA (13) and BMI (14) were as follows:
$BSA(m^2)$=square root (BW[kg] × height [cm]/3600)
$BMI(kg/m^2)$=BW(kg)/height$(m)^2$

Our goal was to develop a simple formula for men and women relating a single factor to the standard BMD. Measured BMD was plotted against age, height, BW, BSA, or BMI, and a formula was developed using simple regression. The significance of the regressions was determined as previously described (15).

Measurement of bone mineral density (BMD). BMD was measured as cross-sectional average pixel density (HU) only in trabecular bone within a circle in the midvertebral core at the bottom of the eleventh thoracic vertebral level (Th11) on preoperative enhanced CT, as previously described (9) (Figure 1a).

Validation set. We then applied our formula to estimate the standard BMD in another 50 healthy donors (25 males and 25 females, aged 22-63 years). The significance of regressions between the estimated BMD by our formula and the measured BMD was determined as previously described (15).

Ethical approval. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent. Informed consent was obtained from all individual participants included in the study.

Statistical analysis. All statistical analyses were performed with JMP statistical software version 13 (SAS Institute Inc., Cary, NC, USA) (16). Continuous variables were expressed as mean±standard deviation (SD), and compared using the nonparametric Wilcoxon test for independent samples. *p*-Values <0.05 were considered statistically significant.

Results

Control set. The median value of BMD was 215.6 HU (25th percentile, 166.6 HU; 75th percentile, 246.9 HU; Figure 1b). Age, body size parameters, and BMD of the donor candidates are shown in Table I. Except for BMI, body size parameters were significantly greater in males compared with females ($p<0.001$).

The measured BMD was not significantly correlated with all of the body size parameters, such as BSA, BW, height, and BMI in both men and women (Figure 2). In contrast, the measured BMD was significantly correlated with age in both males and females (male, $R^2=0.55$, $p<0.0001$; female, $R^2=0.44$, $p<0.0001$; Figure 3a and b). By linear regression analysis to predict the BMD for healthy adults, the following equations were derived from our data of 50 healthy donors (25 males and 25 females):
BMD (HU) for males=308.82-2.49 × Age
BMD (HU) for females=311.84-2.41 × Age

Validation set. Figure 3c and d shows the relationship between measured BMD and estimated BMD, which was calculated using the sex-specific formula in the other cohort of healthy 50 donors (25 males and 25 females). The estimated BMD significantly correlated with the measured BMD in both males and females (male, $R^2=0.68$, $p<0.0001$; female, $R^2=0.49$, $p<0.0001$). The mean difference between the calculated BMD and measured BMD was 2.76±20.54 HU (–46.61 to 31.44) for males and 10.76±24.41 HU (–37.83 to 62.41) for females.

Representative cases. Figure 4 shows two representative cases of osteopenia and non-osteopenia, who underwent living donor liver transplantation (LDLT) because of decompensated liver cirrhosis. Case 1 (Figure 4a) was a 64-year-old male. His physical parameters were as follows: height, 169 cm; weight, 53 kg; BSA, 1.58 m^2; and his BMD, measured by preoperative CT was 108.7 HU. The BMD calculated by our formula was 147.3 HU and the difference between the measured and calculated and BMD was -38.6 HU. Thus, we retrospectively diagnosed that he had osteopenia before LDLT. His Model for End-Stage Liver Disease (MELD) score was 19 points before LDLT, and he received an extended left and caudate lobe graft, weighting 465 g (41.0 % of standard liver weight) from his 31-year-old son. Although the graft functioned well after reperfusion, he died 124 days after LDLT because of bacterial sepsis.

Case 2 (Figure 4b) was a 60-year-old male. His physical parameters were as follows: height, 153 cm; weight, 60 kg; BSA, 1.60 m^2; and his BMD was 261.3 HU. The BMD calculated by our formula was 168.0 HU. The difference between the measured and calculated and BMD was 93.3 HU. Thus, we retrospectively diagnosed that he did not have osteopenia before LDLT. His MELD score was 14 points before LDLT, and he received an extended left and caudate lobe graft, weighting 398 g (35.7% of standard liver weight) from his 31-year-old son. Although he had bacterial sepsis due to cholangitis after LDLT, she recovered and was discharged from hospital 50 days after LDLT. She is alive for 2,407 days after LDLT.

Discussion

This is the first report to demonstrate that BMD values measured by CT were highly correlated with age. Additionally, measurement of BMD was able to accurately estimate osteopenia. A simple and new formula for estimating BMD in each sex was established using data from healthy adults.

Previous studies have found that both skeletal muscle and bone loss are compounded by decreased daily living activities, which results in reduced mechanical loading (4). Previously we demonstrated the measured skeletal muscle area was significantly correlated with BW, height, BMI, and BSA, and

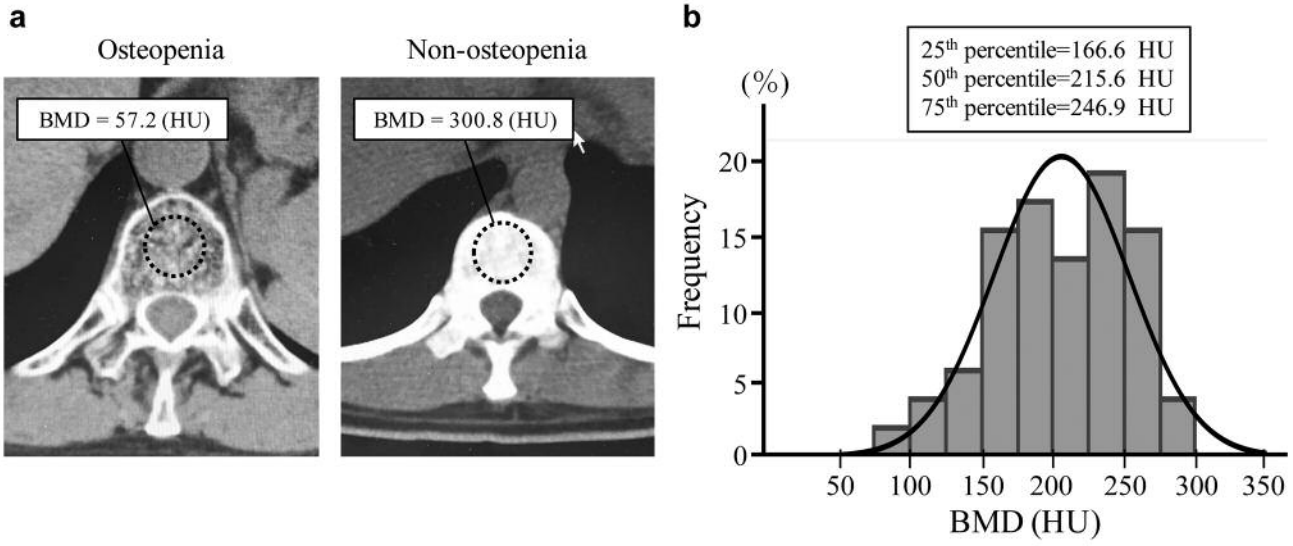

Figure 1. *(a) Measurement of trabecular BMD within a circle in midvertebral core at bottom of 11th thoracic vertebral level (Th11). (b) Distribution of measured BMD among healthy adults (n=50). BMD, Bone mineral density; HU, Hounsfield units.*

Table I. *Body size and bone mineral density in 50 healthy adults.*

Variables	Male (n=25)	Female (n=25)	*p*-Value
Age (years, range)	44.2±13.7 (21-62)	41.0±13.4 (20-64)	0.414
Body height (cm, range)	170.6±4.7 (162-180)	158.7±6.1 (148-177)	0.001
Body weight (kg, range)	65.7±8.7 (51-81)	53.5±9.3 (41-82)	0.001
Body surface area (m^2, range)	1.76±0.13 (1.53-2.00)	1.53±0.16 (1.31-2.01)	0.001
BMI (kg/m^2, range)	22.6±2.7 (18.6-28.0)	21.1±2.4 (16.8-26.2)	0.099
BMD (HU, range)	199.0±45.9 (106.9-288.7)	213.1±48.4 (96.8-294.2)	0.304

BMD, Bone mineral density; BMI, body mass index; HU, Hounsfield units.

that BSA was correlated highest with the skeletal muscle area (15). Sarcopenia, defined by a BSA-dependent formula, has been reviewed as an independent predictor of mortality in severe liver disease such as liver cirrhosis and hepatocellular carcinoma (4, 17). By contrast, as shown in Figure 2, there was no significant correlation between the measured BMD and these body size parameters. However, only age was significantly correlated with higher R^2 values. This suggests that BMD is not associated with individual variations in body size, and each body size is not a reliable predictor of osteopenia. Therefore, we used BMD data to define osteopenia in our validation of the formulae based on age.

In the validation subjects, R^2 was relatively high as shown in Figure 3. The goal of this study was to establish a formula to define osteopenia. Differences between the calculated BMD and estimated BMD were between –46.61 to 31.44 for males and –37.83 to 62.41 for females. Thus, for example, when a patient has a measured BMD of 50 HU for males or 40 HU for females, which is less than the BMD calculated using our formula, the patient is osteopenic. Additional studies are needed to evaluate diagnostic ability, sensitivity, specificity, accuracy, positive predictive value and negative predictive value in patients with liver disease.

Recently, Santos *et al*. (18) reported that low handgrip strength, which indicates sarcopenia, can be a predictor of bone disease linked to liver cirrhosis. They also stated that bone impairment is already present in patients with compensated cirrhosis, and the rates of osteopenia in these patients may be similar to those in findings from the patients with decompensated liver cirrhosis as LT candidates. Considering the relationship between sarcopenia and osteopenia, it is very important to accurately define them, but this also seems very difficult. Regarding the cutoff value of BMD to define osteopenia, Sharma *et al*. (9) demonstrated

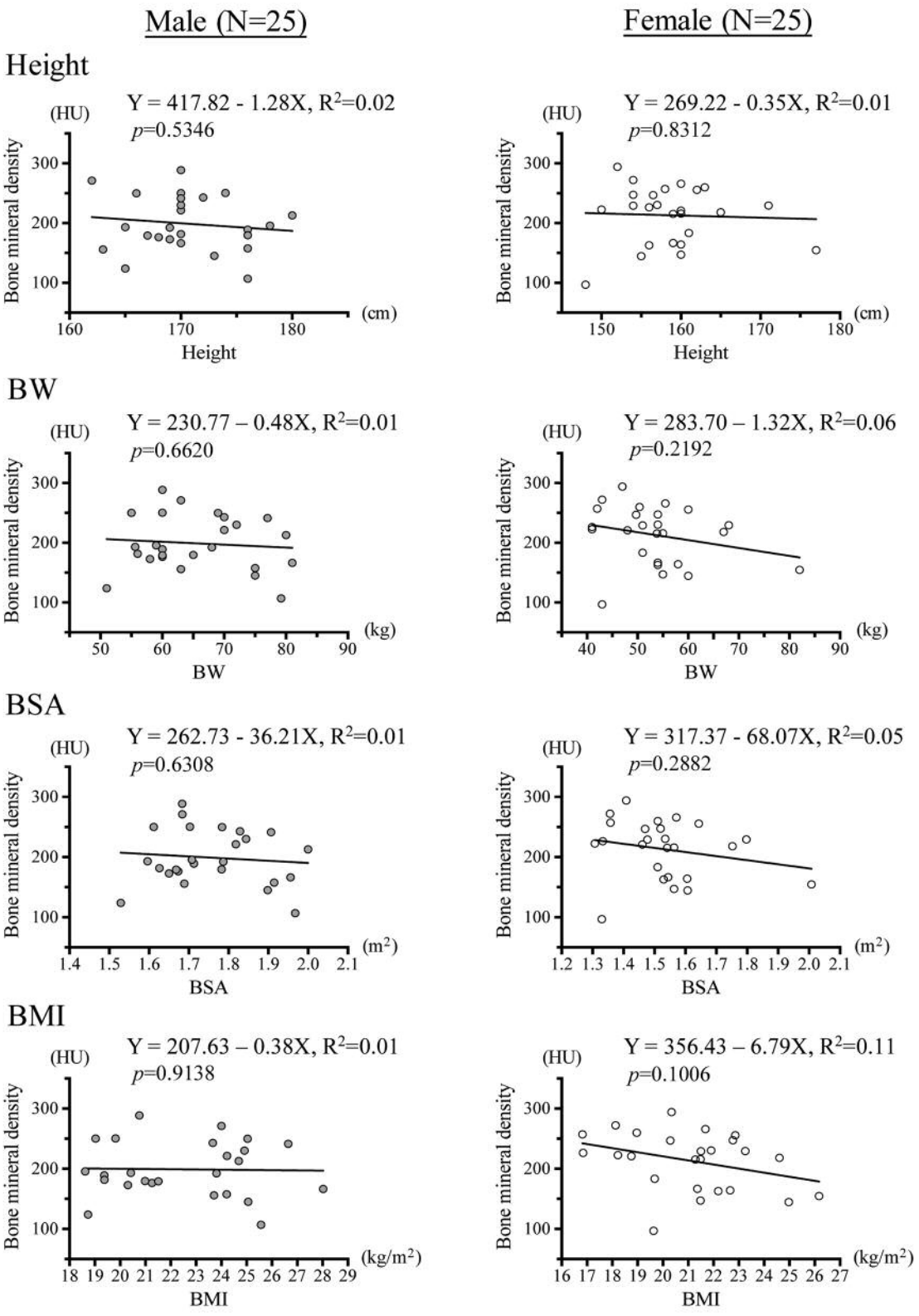

Figure 2. *Relationship between measured BMD and each body size parameter in 50 healthy adults. The measured BMD was not significantly correlated with all body size parameters, such as height, BW, BSA, and BMI in both men (left panel) and women (right panel). BW, Body weight; BSA, body surface area; BMI, body mass index.*

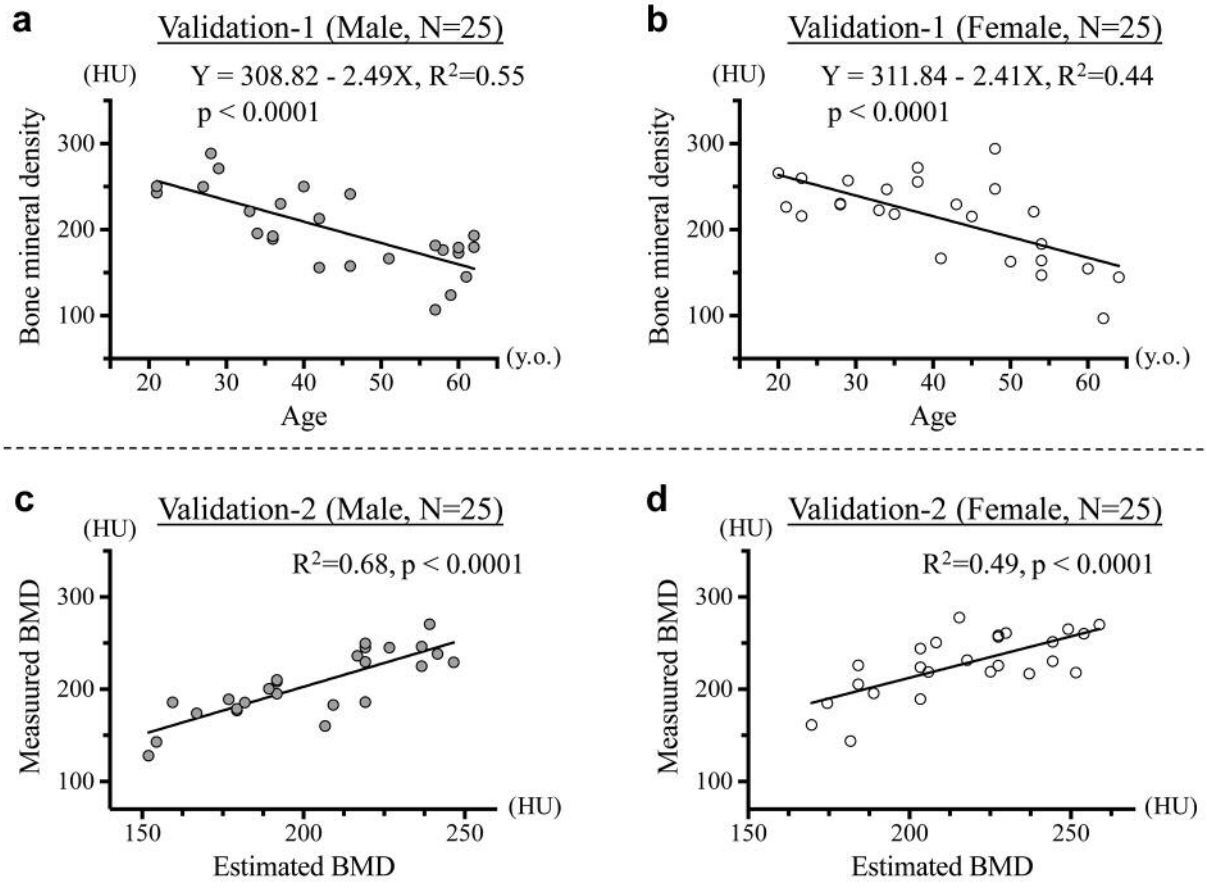

Figure 3. *Relationship between measured BMD and age in 50 healthy adults (a, b). The measured BMD was significantly correlated with age in both males and females (male, R^2=0.55, p<0.0001; female, R^2=0.44, p<0.0001). The BMD in each sex was predicted using the following equations: (a) BMD (HU) for males=308.82 – 2.49 × Age; (b) BMD (HU) for females=311.84-2.41 × Age. Relationship between measured BMD and estimated BMD in each sex in another 50 healthy adults (c, d). The estimated BMD significantly correlated with the measured BMD in both males and females (male, R^2=0.68, p<0.0001; female, R^2=0.49, p<0.0001). BMD, Bone mineral density.*

that the post-LT mortality was about 3-fold higher among those who have BMD <160 HU and, accordingly, they used 160 HU as the cut-off of BMD, regardless of age. However, the results of the present study, indicate that the standard BMD clearly varies with age, even in healthy adults. Therefore, it is not always accurate to define osteopenia by an absolute value cutoff like the method of Sharma *et al*. In this respect, our formula, based on the regression analysis with age correlation for each patient, is feasible for use in definition of osteopenia.

In severe liver disease, for example, liver cirrhosis is a major cause of morbidity and mortality and many efforts have been made to improve the survival and life quality of patients. Osteopenia is a representative complications of severe liver disease (9, 19). Crawford *et al*. (19) demonstrated that the osteoporotic fracture rate in patients with chronic liver disease is approximately twice of that of age-matched, control individuals. Indeed, in cases of impaired liver function, it seems reasonable that bone disease would be more prominent. They estimated the prevalence of osteopenia among these patients as 26-42%, which indicated the strong relationship between impaired liver function and osteopenia. Regarding the hormonal impact, Chen CC *et al*. (20) reported that cirrhosis severity was associated with changes in the serum levels of 25-vitamin D and testosterone, but not with serum levels of osteocalcin or parathyroid hormone. These findings are part of a complex physiopathology involving cirrhosis-related bone disease; however, if a lack of vitamin D is the cause of osteopenia, various complications may be prevented by vitamin D supplementation in these patients. Therefore, it is very important to accurately figure out the presence of osteopenia in patients with severe liver disease. To achieve this goal, comparison of measured BMD to standard BMD is quite feasible.

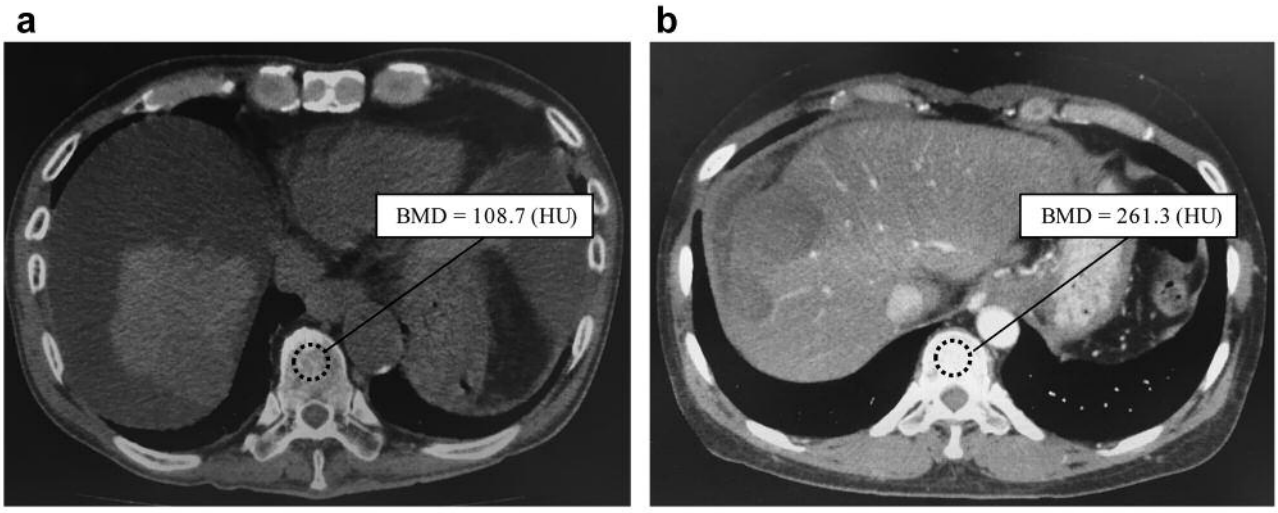

Figure 4. *Two representative cases of osteopenia and non-osteopenia by computed tomography scans. (a) The measured BMD of the patient was 108.7 HU, which was lower than the calculated BMD by our formula of 147.3 HU. He had osteopenia and died 124 days after LDLT because of bacterial sepsis. (b) The measured BMD of the patient was 261.3 HU, which was higher than the calculated BMD by our formula of 168.0 HU. Although she had bacterial sepsis due to cholangitis after LDLT, she recovered and is alive more than 6 years after LDLT.*

In the first discovery step, we used the data from 50 healthy living donors to make the formula for predicting the standard BMD. Next, as a validation step we constructed strong evidence with data from another 50 healthy donors to compensate the small number of cases. Therefore, the predictive formula for the standard BMD with high reproducibility could be created.

In conclusion, we analyzed BMD using CT data from healthy adults and found that age was significantly correlated with BMD. Osteopenia can be defined by the difference between measured and calculated data using the newly-established formula.

Funding

This study was supported by the following grants: the Program for Basic and Clinical Research on Hepatitis from the Japan Agency for Medical Research and Development, AMED (Numbers 17kf02101077h0001 and 17fk0210305h0003) and JSPS KAKENHI, a Grant-in-Aid from the Ministry of Health, Labour and Welfare, Japan (Numbers JP-16K10577 and JP-16K06432). The funding sources had no role in the collection, analysis, or interpretation of the data, or in the decision to submit the article for publication.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

The Authors thank Rebecca Tollefson, DVM, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

References

1 Raisz LG: Pathogenesis of osteoporosis: Concepts, conflicts, and prospects. J Clin Invest *115*: 3318-3325, 2005.

2 Lumachi F, Basso SMM, Camozzi V, Spaziante R, Ubiali P and Ermani M: Bone mineral density as a potential predictive factor for luminal-type breast cancer in postmenopausal women. Anticancer Res *38*: 3049-3054, 2018.

3 Rosario M, Takeuchi A, Yamamoto N, Hayashi K, Miwa S, Higuchi T, Abe K, Taniguchi Y, Aiba H, Tanzawa Y, Murakami H and Tsuchiya H: Pathogenesis of osteosclerotic change following treatment with an antibody against rankl for giant cell tumour of the bone. Anticancer Res *37*: 749-754, 2017.

4 Harimoto N, Shirabe K, Yamashita YI, Ikegami T, Yoshizumi T, Soejima Y, Ikeda T, Maehara Y, Nishie A and Yamanaka T: Sarcopenia as a predictor of prognosis in patients following hepatectomy for hepatocellular carcinoma. Br J Surg *100*: 1523-1530, 2013.

5 Wu K, Feskanich D, Fuchs CS, Willett WC, Hollis BW and Giovannucci EL: A nested case control study of plasma 25-hydroxyvitamin d concentrations and risk of colorectal cancer. J Natl Cancer Inst *99*: 1120-1129, 2007.

6 Lee JE, Li H, Giovannucci E, Lee IM, Selhub J, Stampfer M and Ma J: Prospective study of plasma vitamin b6 and risk of colorectal cancer in men. Cancer Epidemiol Biomarkers Prev *18*: 1197-1202, 2009.

7 Peterlik M and Cross HS: Dysfunction of the vitamin d endocrine system as common cause for multiple malignant and other chronic diseases. Anticancer Res *26*: 2581-2588, 2006.

8 Miyazawa Y, Sekine Y, Syuto T, Nomura M, Koike H, Matsui H, Shibata Y, Ito K and Suzuki K: Evaluation of bone turnover/quality markers and bone mineral density in prostate cancer patients receiving androgen deprivation therapy with or without denosumab. Anticancer Res *37*: 3667-3671, 2017.

9 Sharma P, Parikh ND, Yu J, Barman P, Derstine BA, Sonnenday CJ, Wang SC and Su GL: Bone mineral density predicts posttransplant survival among hepatocellular carcinoma liver transplant recipients. Liver Transpl *22*: 1092-1098, 2016.
10 Pickhardt PJ, Pooler BD, Lauder T, del Rio AM, Bruce RJ and Binkley N: Opportunistic screening for osteoporosis using abdominal computed tomography scans obtained for other indications. Ann Intern Med *158*: 588-595, 2013.
11 Chen JH, Chen YC, Tsai MK, Chiou JM, Lee WC, Tsao CK, Tsai KS and Chie WC: Predicting the risk of osteopenia for women aged 40-55 years. J Formos Med Assoc *116*: 888-896, 2017.
12 Kraemer B, Rothmund R, Banys M, Krawczyk N, Solomayer EF, Mack C, Wallwiener D and Fehm T: Impaired bone microenvironment: Correlation between bone density and presence of disseminated tumor cells. Anticancer Res *31*: 4423-4428, 2011.
13 Mosteller RD: Simplified calculation of body-surface area. N Engl J Med *317*: 1098, 1987.
14 Methods for voluntary weight loss and control. Nih technology assessment conference panel. Ann Intern Med *116*: 942-949, 1992.
15 Yoshizumi T, Shirabe K, Nakagawara H, Ikegami T, Harimoto N, Toshima T, Yamashita Y, Ikeda T, Soejima Y and Maehara Y: Skeletal muscle area correlates with body surface area in healthy adults. Hepatol Res *44*: 313-318, 2014.
16 Toshima T, Shirabe K, Fukuhara T, Ikegami T, Yoshizumi T, Soejima Y, Ikeda T, Okano S and Maehara Y: Suppression of autophagy during liver regeneration impairs energy charge and hepatocyte senescence in mice. Hepatology *60*: 290-300, 2014.
17 Masuda T, Shirabe K, Ikegami T, Harimoto N, Yoshizumi T, Soejima Y, Uchiyama H, Ikeda T, Baba H and Maehara Y: Sarcopenia is a prognostic factor in living donor liver transplantation. Liver Transpl *20*: 401-407, 2014.
18 Santos LA, Lima TB, Augusti L, Franzoni Lde C, Yamashiro Fda S, Bolfi F, Nunes Vdos S, Dorna Mde S, de Oliveira CV, Caramori CA, Silva GF and Romeiro FG: Handgrip strength as a predictor of bone mineral density in outpatients with cirrhosis. J Gastroenterol Hepatol *31*: 229-234, 2016.
19 Crawford BA, Labio ED, Strasser SI and McCaughan GW: Vitamin d replacement for cirrhosis-related bone disease. Nat Clin Pract Gastroenterol Hepatol *3*: 689-699, 2006.
20 Chen CC, Wang SS, Jeng FS and Lee SD: Metabolic bone disease of liver cirrhosis: Is it parallel to the clinical severity of cirrhosis? J Gastroenterol Hepatol *11*: 417-421, 1996.

Received September 14, 2018
Revised September 21, 2018
Accepted September 28, 2018