

Skin advanced glycation end-product accumulation is negatively associated with calcaneal osteo-sono assessment index among non-diabetic adult Japanese men

H. Momma · K. Niu · Y. Kobayashi · L. Guan ·
M. Sato · H. Guo · M. Chujo · A. Otomo · C. Yufei ·
H. Tadaura · T. Saito · T. Mori · T. Miyata · R. Nagatomi

Received: 13 April 2011 / Accepted: 10 August 2011 / Published online: 8 September 2011
© The Author(s) 2011. This article is published with open access at Springerlink.com

Abstract

Summary This study aims to determine the relationship between advanced glycation end-product (AGE) accumulation in skin tissue and bone strength, assessed by quantitative ultrasound, among healthy adult Japanese men. The results of the study suggest that men with higher AGE accumulation in skin tissue have a lower osteo-sono assessment index.

Introduction AGE accumulate in bone collagen with age and diabetes and decrease the mechanical properties of bone. Although increased AGE levels are associated with fractures among diabetic patients and elderly women, it is unclear whether a relationship between increased AGE levels and bone strength is present in apparently healthy adult males. The aim of this study was to determine the relationship between AGE accumulation in tissue and the mechanical properties of bone among adult Japanese men, using quantitative ultrasound as a surrogate measure of the latter.

Methods Skin autofluorescence (AF), which is a noninvasive method for measuring tissue AGEs, and osteo-sono assessment index (OSI), which is determined by quantitative ultrasound, were measured in 193 adult Japanese men (median age 43 years; interquartile range 37.0–55.0 years).

Results Adjusted for age, BMI, calcium intake, physical activity, smoking status, and education level, log-transformed skin AF had a negative association with log-transformed OSI ($\beta=-0.218$, $P<0.01$). Adjusted geometric means (95% CI) for OSI across the tertiles of skin AF were 2.81 (2.75–2.87) for the lowest tertile, 2.81 (2.74–2.87) for the middle tertile, and 2.66 (2.61–2.73) for the highest tertile; thus, OSI for the highest skin AF appeared to be 5.0% lower than that for the lowest and middle skin AF tertiles ($P<0.01$).

Conclusion Among apparently healthy adult Japanese men, those with higher skin AF had a lower OSI, indicating a relationship between AGE accumulation and bone strength. A long-term prospective study is required to clarify the causality.

H. Momma · K. Niu · T. Saito · R. Nagatomi (✉)
Division of Biomedical Engineering for Health and Welfare,
Tohoku University Graduate School of Biomedical Engineering,
2-1 Seiryomachi, Aoba-ku,
Sendai 980-8575, Japan
e-mail: nagatomi@med.tohoku.ac.jp

Y. Kobayashi · L. Guan · M. Sato · H. Guo · M. Chujo ·
A. Otomo · C. Yufei · H. Tadaura
Department of Medicine and Science in Sports and Exercise,
Tohoku University Graduate School of Medicine,
Sendai, Japan

T. Mori · T. Miyata
United Centers for Advanced Research and Translational
Medicine, Tohoku University Graduate School of Medicine,
Sendai, Japan

Keywords Advanced glycation end-products · Bone quality · Bone strength · Carbonyl stress · Oxidative stress · Quantitative ultrasound

Introduction

Osteoporosis is a critical public health problem due to its association with bone fragility and susceptibility to fracture [1]. According to the U.S. National Institutes of Health, osteoporosis is defined as a systemic skeletal disorder characterized by compromised bone strength [2]. Bone strength is not only determined by measures of bone density, such as mass and mineral density, but also by bone quality,

including microarchitecture, turnover, accumulation of microdamage, mineralization, and quality of collagens [2, 3]. Interestingly, patients with type 2 diabetes have an increased risk of fracture despite normal or high bone mineral density (BMD) compared with non-diabetic controls, suggesting poorer bone quality in diabetic patients [4].

Accumulation of advanced glycation end-products (AGEs), which are often found in diabetic patients, in bone collagen has been proposed as a factor responsible for reducing bone strength with aging [5], diabetes [6, 7], and osteoporosis [8–10]. AGEs are a diverse class of compounds resulting from non-enzymatic reactions between glucose and proteins. A common consequence of AGE formation is covalent cross-linking, mostly to proteins including collagen. Accumulation of AGEs in bone collagen decreases the mechanical properties of bone collagen [11, 12]. In rats, an increase of AGE content in bone decreases the mechanical properties of bone despite normal BMD [6]. In humans, the AGE concentration of cortical bone shows a significant increase with age and is negatively correlated with osteoporotic index (Singh score) [5]. Moreover, the AGE content in bone is higher in patients with hip fracture than in subjects without fractures [10].

In a population study, Shiraki et al. demonstrated that a high level of urinary pentosidine, a major AGE in vivo, was an independent risk factor for osteoporotic vertebral fractures in elderly women [13]. Schwartz et al. reported that urinary pentosidine content was associated with increased fracture incidence in older adults with diabetes [14]. The subjects of these studies were older adults who had an increased risk of life-related diseases, such as diabetes and osteoporosis. However, AGEs may accumulate before the onset of diabetes and even at a younger age. In non-diabetic Japanese subjects, serum AGE levels were independently correlated with insulin resistance, which may gradually cause diabetes [15]. Pentosidine content in bone or serum increased with advancing age [5]. Given that bone strength commonly peaks when a person is in his/her 20s and then gradually declines with advancing age, AGE accumulation may be associated with bone strength, if not with fractures, preclinically. Moreover, in men, the lifetime risk of any osteoporotic fracture has been assessed as being within the range 13–22% [1], so osteoporosis is no longer a problem only for women and the elderly. Greater AGE accumulation may potentially be related to poorer bone strength in apparently healthy adult men.

Thus, in this study, we examined the association between skin autofluorescence (AF), which is associated with skin accumulation of AGEs, including pentosidine [16], and quantitative ultrasound examination of calcaneal bone, which correlates with mechanical properties of the bone

and may have a predictive value for hip fractures in men [17], among apparently healthy adult men. We hypothesized that skin AF would have a negative association with quantitative ultrasound among adult men.

Methods

Study participants

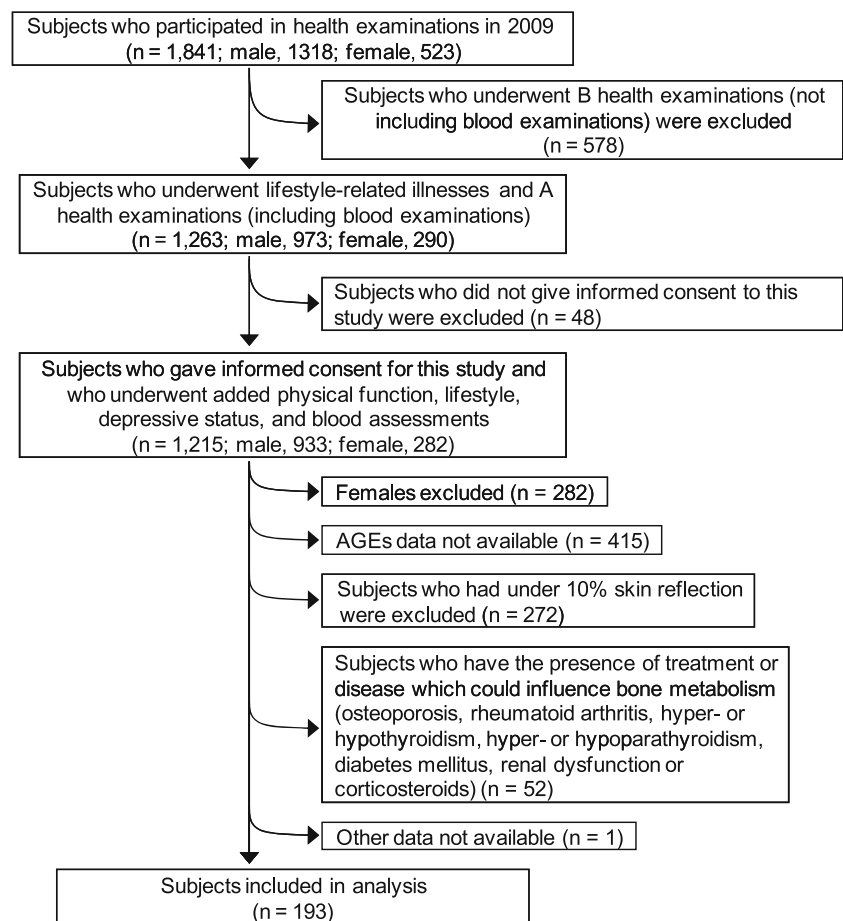
The study participants consisted of adult male employees enrolled in a prospective study of risk factors for lifestyle-related illnesses or health status in Japan. Participants received annual health examinations including anthropometric measurements, hematological examinations, and, in 2009, an additional assessment including the accumulation of AGEs in skin and quantitative ultrasound examination of calcaneal bone. This study was carried out during the first week (from Monday to Friday) of August. The details of this study have been described elsewhere [18, 19].

The sample selection process is described in Fig. 1. In 2009, 1,263 participants had undergone health examinations for lifestyle-related illnesses. Of these, 1,215 (933 men) participated in our survey and provided their informed consent for data analysis (response rate, 96.2%). Those who underwent skin AF measurement were randomly selected ($n=518$). Two hundred seventy-two (272) participants who had a low skin reflection (<10%) were excluded (details are provided in the [Skin autofluorescence](#) section below). Furthermore, we excluded 52 men who had and/or were receiving treatment for a disease that could influence bone metabolism (osteoporosis, rheumatoid arthritis, hyper- or hypothyroidism, hyper- or hypoparathyroidism, diabetes mellitus, renal dysfunction, or corticosteroid use); one man was excluded because of incomplete data. As a result, 193 men were included in the present study. None had a history of vertebral fractures. The protocol of this study was approved by the Institutional Review Board of the Tohoku University Graduate School of Medicine.

Skin autofluorescence

AGE accumulation in skin tissue was assessed on the basis of skin AF, using an AF reader (AGE Reader; DiagnOptics, Groningen, The Netherlands), as described previously [16]. The AGE Reader consists of a tabletop box equipped with an excitation light source. Each measurement took approximately 30 s to complete and was performed by an independent observer. Excitation light of 300–420-nm wavelength was projected onto the skin surface through a 1-cm² hole. The intensity of light emitted from the skin at wavelengths between 420 and 600 nm was measured with a

Fig. 1 Flow chart of the sample selection process AGEs, advanced glycation end-products



spectrometer via a glass fiber. Skin AF was calculated by dividing the mean value of the emitted light intensity per nanometer between 420 and 600 nm by the mean value of the excitation light intensity per nm between 300 and 420 nm; the result was expressed in arbitrary unit (AU) and multiplied by 100 for easier evaluation. The intra- and inter-assay coefficients of variation for AGE reader measurement were 2.9–1.8%, respectively.

All AF measurements were performed at room temperature on the volar side of the lower right arm, approximately 10–15 cm below the elbow fold, with the participants in a seated position. Care was taken to perform the measurement at a normal skin site without visible vessels, scars, lichenification, or other skin abnormalities. The arm of each subject was covered with a black cloth to avoid any influence of external light during the measurement. Because creams and sunscreens can affect skin AF measurement [20], we asked each participant whether they applied creams or sunscreens on their arms when skin AF was measured. No participants applied any creams or sunscreens. Since skin pigmentation influences AF measurements, particularly when skin reflection is below 10%, AF values were not used if the skin reflection was below 10% [21].

Quantitative ultrasound assessment of the calcaneus

Quantitative ultrasound assessment of the calcaneus was performed using an ultrasound system (AOS-100; Aloka Co. Ltd., Tokyo, Japan). The AOS-100 measured the speed of sound (SOS) as an index of bone density and the transmission index (TI) as an index of bone structure. The osteo-sono assessment index (OSI) was calculated using the following formula: $OSI = TI \times SOS^2$. OSI provides information on bone stiffness in a radiation-free, convenient, and portable manner. Because this study was conducted along with annual health examinations, a rapid and simple measurement was prerequisite. The intra- and inter-assay coefficients of variation for the OSI were 1.1–0.8%, respectively.

Assessment of other variables

Blood samples were drawn from the antecubital vein, with minimal tourniquet use, while the subjects were seated. Specimens were collected in siliconized vacuum glass tubes containing sodium fluoride for fasting blood glucose and no additives for lipid analyses. Fasting blood glucose concentration was measured by enzymatic methods (Eerotec,

Tokyo, Japan). The triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) concentrations were measured by enzymatic methods using appropriate kits (Sekisui Medical, Tokyo, Japan).

Depressive symptoms were assessed according to the Japanese version [22] of the Self-Rating Depression Scale (SDS). Participants were considered as depressive when the SDS score was 45 or more [23].

Blood pressure (BP) in the left upper arm was measured twice using an automatic device (YAMASU605P; Kenzmedico Co. Ltd., Saitama, Japan) following a 5-min rest in a seated position. The mean value was used as the BP value.

Anthropometric parameters (height and body weight) were recorded using a standard protocol. Body mass index (BMI) was calculated as weight (kilogram) divided by height in meters squared. Sociodemographic variables, including age and educational level, were also assessed. Educational level was assessed by determining the final grade level and was divided into two categories: lower than college level and college level and above. History of physical illness and current medication use were evaluated on the basis of “yes” or “no” responses to questions. History of fractures was obtained from a questionnaire owing to the unavailability of clinical data and was divided into two categories: those who had a history of lower extremity fractures and those who did not. Information on smoking status (never, former, or currently smoking and Brinkman index), alcohol-drinking status (never, ≥ 1 day/week, or 7 days/week), and occupation (desk based or not), was obtained from a questionnaire survey. Levels of daily physical activity (PA) were estimated using the International Physical Activity Questionnaire (Japanese version) [24], and categorized into tertiles (low, middle, and high). Calcium, vitamin D, and alcohol intake were estimated using a brief self-administered dietary history questionnaire [25]. A diagnosis of metabolic syndrome (MS) was defined according to the modified Japanese criteria (defined by the Japanese Society for the Study of Obesity) [26].

Statistical analysis

All statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA).

In this study, because the distribution of all continuous variables except for systolic BP was not normal, the natural logarithm was applied to normalize the data before statistical analysis (analysis of variance [ANOVA], analysis of covariance [ANCOVA], and linear regression analysis). Descriptive statistics were computed for each variable by using logistic regression analysis and ANOVA. Descriptive data are represented as the median (interquartile range) for non-adjusted continuous variables, geometric means (95%

confidence interval [CI]) for adjusted continuous variables, and percentages for categorical variables. Linear regression analysis was used to examine the relationship between log-transformed skin AF and other factors with log-transformed OSI. All variables included in Table 1 were analyzed by univariable linear regression. Variables that were at a level of significance of $P < 0.10$ in univariate analyses were included in the multivariate models. Multiple linear regression analysis was performed to determine the independent relationship of variables with log-transformed OSI. ANCOVA using log-transformed OSI as the dependent variable and the tertiles of skin AF as independent variables was performed with adjustment for the same variables as in the multiple linear regression models. Bonferroni-corrected P values were used for comparisons between groups differing in skin AF. All tests for statistical significance were two-sided, and $P < 0.05$ was defined as statistically significant.

Results

Characteristics of the 193 study participants are shown in Table 1. Overall, median (interquartile range) OSI was 2.75 (2.59–2.93), and skin AF was 1.96 (1.78–2.14) AU. Median age was 45.0 years.

In the univariate analyses (Table 2), log-transformed OSI was significantly associated with age, BMI, calcium intake, high PA, former smoker, and skin AF. The association of log-transformed OSI with waist circumference, education level (college level and above), and MS were borderline significance, and there was no association of log-transformed OSI with fasting blood glucose, TG, LDL-C, HDL-C, BP, vitamin D intake, middle PA, current smoker, drinking status, depressive symptoms ($\text{SDS} \geq 45$), desk work, and leg fracture. Among current smokers, Brinkman index was associated with OSI ($r = -0.16$, $P = 0.04$, data not shown).

To determine whether skin AF was independently associated with OSI, we performed a multiple linear regression analysis using skin AF and other variables associated with OSI in the univariate analyses (Table 3). Although waist circumference had a tendency to associate with OSI in the univariate model, waist circumference was not included in the multivariate model since it was strongly correlated with BMI. After adjustment for age, BMI, calcium intake, PA level, smoking status, education level, and MS, log-transformed skin AF had a negative association with log-transformed OSI ($\beta = -0.218$, $\text{SE} = 0.069$, $P < 0.01$). Table 4 shows the relationship of the tertiles of skin AF with log-transformed OSI using ANCOVA. The adjusted geometric mean (95% CI) of log-transformed OSI across the tertiles of skin AF was 2.81 (2.75–2.87) for the lowest tertile, 2.81 (2.74–2.87) for the middle tertile,

Table 1 Characteristics of participants ($n=193$)

Characteristic	Median (interquartile range) or percentage, %
Age (years)	45.0 (37.0–55.0)
BMI (kg/m^2)	23.7 (21.9–25.8)
Waist circumference (cm)	85.0 (79.0–91.0)
SBP (mm Hg)	128.0 (118.0–138.0)
DBP (mm Hg)	80.0 (74.0–90.0)
Fasting blood glucose (mg/dL)	93.0 (88.0–100.0)
TG (mg/dL)	128.0 (77.0–183.0)
LDL-C (mg/dL)	121.0 (103.0–140.0)
HDL-C (mg/dL)	52.0 (43.0–58.0)
Calcium intake (mg/day \cdot 2,000 kcal)	460.1 (349.1–606.8)
Vitamin D intake (mg/day \cdot 2,000 kcal)	10.8 (7.1–16.0)
High PA (median values, 48.0 METs h/week)	33.2
Middle PA (median values, 12.0 METs h/week)	33.2
Smoking status	
Current	40.3
Former	13.7
Drinking status	
7 drinks/week	28.0
≥ 1 drink(s)/week	55.9
Depressive symptoms (SDS ≥ 45)	29.9
Education (\geq college)	38.4
Desk work	79.6
Leg fracture	16.6
MS (JASSO)	22.7
Skin AF	1.96 (1.78–2.14)
OSI	2.75 (2.59–2.93)

BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, PA physical activity, SDS Self-rating Depression Scale, MS metabolic syndrome, JASSO Japanese Society for the Study of Obesity, AF autofluorescence, OSI osteo-sono assessment index

and 2.66 (2.61–2.73) for the highest tertile; thus, participants in the highest tertile had 5.0% lower OSI than those in the lowest and middle tertiles (Bonferroni-corrected P value <0.01).

Discussion

The present study examined the relationship between skin AF associated with AGE accumulation and OSI, a quantitative ultrasound measure, among non-diabetic adult Japanese men. Consistent with our hypothesis, our results showed that levels of skin AF were independently associated with OSI, suggesting that participants with higher skin AF had lower OSI.

In previous population studies, the relationship between AGE accumulation and fracture risk has been controversial. Some studies reported that there was no association between urinary pentosidine and fracture risk after adjustment in non-diabetic older Caucasian [14] and among postmenopausal Caucasian women [27]. On the other hand, in elderly Japanese women, a high level of urinary

pentosidine was an independent risk factor for osteoporotic vertebral fractures [13]. Possibly in line with these findings, we found a negative association between skin AF with OSI among adult Japanese men after adjustment for potential confounders, given that lower OSI may lead to higher fracture risk. Although the reasons for this discrepancy are unknown, racial differences may potentially explain the inconsistent results of the studies. While Japanese have twice the incidence of the methylenetetrahydrofolate reductase polymorphism (C677T) compared with Caucasians, Japanese subjects are predisposed to mild hyperhomocysteinemia [28–30]. Indeed, hyperhomocysteinemia caused a reduction in bone toughness through the accumulation of pentosidine in bone in rabbit models [31]. Other explanation could be diet, which is a major source of exogenous AGEs [32]. AGEs are especially high in Western diet, since heat-treated process enhances the formation of AGEs [32], and the concentration of urinary pentosidine in Caucasian women [27] was more than twofold greater than that in Japanese women [13]. Therefore, Japanese who may be ingesting less dietary AGE might be more susceptible to the adverse effect of AGE accumulation.

Table 2 Univariate linear regression models of skin AF and other factors with OSI

Characteristic	β	<i>P</i> value
Age (years)	-0.26	<0.01
BMI (kg/m ²)	0.20	<0.01
Waist circumference (cm)	0.13	0.06
SBP (mm Hg)	0.03	0.67
DBP (mm Hg)	0.01	0.91
Fasting blood glucose (mg/dL)	-0.10	0.16
TG (mg/dL)	-0.10	0.92
LDL-C (mg/dL)	0.03	0.72
HDL-C (mg/dL)	-0.01	0.85
Calcium intake (mg/day·2,000 kcal)	0.15	0.03
Vitamin D intake (mg/day·2,000 kcal)	0.03	0.64
High PA (median values, 48.0 METs h/week) ^a	0.15	0.03
Middle PA (median values, 12.0 METs h/week) ^a	-0.07	0.30
Smoking status ^b		
Current	-0.03	0.69
Former	-0.15	0.03
Drinking status ^c		
7 drinks/week	-0.06	0.42
≥1 drinks/week	0.09	0.18
Depressive symptoms (SDS≥45)	-0.05	0.49
Education (≥college)	0.12	0.07
Desk work	0.06	0.42
Leg fracture	0.08	0.22
MS (JASSO)	0.13	0.05
Skin AF	-0.25	<0.01

OSI osteo-sono assessment index, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, PA physical activity, SDS Self-rating Depression Scale, MS metabolic syndrome, JASSO Japanese Society for the Study of Obesity, AF autofluorescence
^aReference category is low PA
^bReference category is never
^cReference category is ≤1 drink/week

Skin AF measurement is a noninvasive, rapid, and highly reproducible method, which effectively measures tissue AGE accumulation. This method has been validated to correspond to specific AGE skin levels, including pentosidine [16]. As for the clinical significance of skin AF measurement, however, we still have a limited number of prospective studies in which Skin AF was shown to predict developments of diabetic complications [33], and was associated with all-cause mortality [34] in type 2 diabetes in a prospective study with a follow-up period of 3.1 years. Therefore, more prospective studies with larger

sample size and longer follow-up period are necessary to establish its clinical significance.

Table 3 Multiple linear regression models of log-transformed skin AF with log-transformed OSI

Characteristic	β	SE	<i>P</i> value
Participants (<i>n</i> =193)			
Log-skin AF	-0.218	0.069	<0.01

Adjusted for age, body mass index, calcium intake, physical activity level, smoking status, education level, and metabolic syndrome
 AF autofluorescence, OSI osteo-sono assessment index, SE standard error

Table 4 Relationship of the tertile of skin autofluorescence (AF) with log-transformed OSI among adult Japanese men

Range (unit, AU)	Tertiles of skin AF		
	Low (1.28–1.82)	Middle (1.82–2.05)	High (2.05–2.88)
Number of participants	65	64	64
Crude	2.83 (2.76–2.90)	2.78 (2.71–2.85)	2.68 (2.61–2.74)*
Adjusted ^a	2.81 (2.75–2.87)	2.81 (2.74–2.87)	2.66(2.61–2.73)**

Data are geometric means (95% confidence interval). Unit of leg extension power is watts per kilogram

Analysis of variance or analysis of covariance

* *P*<0.01; significantly different from lowest skin autofluorescence tertile (Bonferroni correction)

** *P*<0.01; significantly different from middle skin autofluorescence tertile (Bonferroni correction)

^a Adjusted for age, body mass index, calcium intake, physical activity level, smoking status, education level, and metabolic syndrome

Sell et al. have shown an exponential increase in pentosidine accumulation across the age in skin collagen [35]. In a separate study, Odetti et al. have shown a similar exponential increase in pentosidine accumulation across the age in bone collagen [5]. Interestingly, the level of pentosidine per unit collagen is higher in the bone as compared to the skin. This difference well corresponds to the result obtained in a cadaver study in which post-mortem bodies of human were analyzed [36]. They showed that pentosidine level per milligram of collagen was more than 60% higher in the bone tissue as compared to the skin tissue. Taken together, skin and bone pentosidine levels are likely to have a positive correlation. Further study is necessary to establish this relationship, but we believe that skin AF may not only correspond to skin pentosidine accumulation, but also bone pentosidine accumulation. In rats, the accumulation of pentosidine in bone was significantly associated with the reduction of bone stiffness [7]. Although the cause–effect relationship cannot be established in this cross-sectional design, we believe that skin AF may be associated with bone strength. Further prospective study is, therefore, required to establish the prospective value of skin AF on bone strength.

In the present study, we used OSI as an index of bone strength. Although OSI is not widely used to assess bone strength, quantitative ultrasound (QUS) parameters including OSI may reflect not only bone mass but also bone quality. A previous study found that impaired bone mechanical properties in diabetic rats coincided with impaired enzymatic cross-link formation and increases in glycation-induced pentosidine, despite the lack of reduction in BMD [7], therefore, it is possible that AGE accumulation may more clearly be associated with OSI rather than BMD which measures bone density. In this study, OSI was 5.0% lower for the highest skin AF compared with the lowest and middle skin AFs after adjustment for confounders. Njeh et al. showed that patients with hip fractures had 8.0% lower OSI compared with control subjects [37]. Therefore, the influence of preclinical AGE accumulation in bone tissue may not be negligible.

Our finding is consistent with the observation in *in vitro* studies that increased AGE level in bone collagen reduces bone mechanical properties [11, 12]. AGE accumulation significantly alters the quantity and morphology of microdamage and results in reduced fracture resistance [38]. Moreover, in spontaneously diabetic rats, decreased mechanical properties of femoral bone are accompanied by increased accumulation of pentosidine, and the pentosidine content is significantly associated with the mechanical properties of bone [6]. Indeed, in several studies, patients with hip fractures had higher bone pentosidine content [9, 10] or serum AGEs [8] compared with subjects without fractures.

In addition to the adverse effects of AGEs on the material properties of bone collagen, AGE accumulation may potentially influence bone cells. AGE-modified bone collagen has detrimental effects on osteoblastic function [7, 39]. The effects of AGEs on osteoclastic bone resorption are controversial. Receptor-of-AGE (RAGE) knockout mice have significantly higher bone mechanical strength, probably due to decreased number of osteoclasts compared with wild-type mice [40]. Furthermore, Miyata et al. showed that AGEs increased the number of resorption pits in cultured mouse bone cells as well as when AGE-accumulated bone particles were implanted subcutaneously in rats [41]. In contrast, Valcourt et al. reported that bone resorption was inhibited in an *in vitro* study using rabbit and human mature osteoclasts seeded on AGE-modified slices [42]. AGEs also inhibited the proliferation of human mesenchymal stem cells and cognate differentiation into bone [43].

Although there was no association between smoking status and OSI in this study, Brinkman index was negatively associated with OSI among current smokers. Therefore, smoking may have harmful effect on bone strength among healthy adult men. As for drinking status, we found no association with OSI. A previous study reported that, among Korean men, four to seven cups of soju (the most popular liquor in Korea) is associated with the risk of reduced QUS parameters [44]. When the amount of alcohol intake in our population was converted to alcohol amount per a cup of soju, only less than 20% of our participants drank the amount of alcohol corresponded to four or more cups of soju (data not shown). Thus, it is possible that the alcohol intake in our study was small in the previous study [44].

This study has some limitations. First, although we adjusted for confounders such as lifestyle factors and disease, we could not exclude the possibility that bone strength was affected by other factors associated with lifestyle or disease. Moreover, because this study was a cross-sectional study, we could not conclude whether AGE accumulation in skin tissue reduced bone strength. A larger population-based prospective study should be performed to further confirm the causal relationship between skin AGE accumulation and bone strength.

In conclusion, in apparently healthy adult Japanese men, skin AF was independently associated with OSI, suggesting that the participants with higher skin AF had a lower OSI. Further studies are needed to confirm the causal relationship between skin AGE accumulation and bone strength.

Acknowledgments We gratefully acknowledge all the subjects participating in our study and the Sendai Oroshisho Center for allowing us to perform the study. This work was supported by “Knowledge Cluster Initiative” from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Conflicts of interest None.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Johnell O, Kanis J (2005) Epidemiology of osteoporotic fractures. *Osteoporos Int* 16(Suppl 2):S3–S7
- Anonymous (2001) Osteoporosis prevention, diagnosis, and therapy. *JAMA* 285:785–795
- Viguet-Carrin S, Garnero P, Delmas PD (2006) The role of collagen in bone strength. *Osteoporos Int* 17:319–336
- Schwartz AV, Sellmeyer DE, Ensrud KE, Cauley JA, Tabor HK, Schreiner PJ, Jamal SA, Black DM, Cummings SR (2001) Older women with diabetes have an increased risk of fracture: a prospective study. *J Clin Endocrinol Metab* 86:32–38
- Odetti P, Rossi S, Monacelli F, Poggi A, Cirmigliaro M, Federici M, Federici A (2005) Advanced glycation end products and bone loss during aging. *Ann N Y Acad Sci* 1043:710–717
- Katayama Y, Akatsu T, Yamamoto M, Kugai N, Nagata N (1996) Role of nonenzymatic glycosylation of type I collagen in diabetic osteopenia. *J Bone Miner Res* 11:931–937
- Saito M, Fujii K, Mori Y, Marumo K (2006) Role of collagen enzymatic and glycation induced cross-links as a determinant of bone quality in spontaneously diabetic WBN/Kob rats. *Osteoporos Int* 17:1514–1523
- Hein G, Wiegand R, Lehmann G, Stein G, Franke S (2003) Advanced glycation end-products pentosidine and N epsilon-carboxymethyllysine are elevated in serum of patients with osteoporosis. *Rheumatology* 42:1242–1246
- Saito M, Fujii K, Marumo K (2006) Degree of mineralization-related collagen crosslinking in the femoral neck cancellous bone in cases of hip fracture and controls. *Calcif Tissue Int* 79:160–168
- Saito M, Fujii K, Soshi S, Tanaka T (2006) Reductions in degree of mineralization and enzymatic collagen cross-links and increases in glycation-induced pentosidine in the femoral neck cortex in cases of femoral neck fracture. *Osteoporos Int* 17:986–995
- Garnero P, Borel O, Gineyts E, Duboeuf F, Solberg H, Bouxsein ML, Christiansen C, Delmas PD (2006) Extracellular post-translational modifications of collagen are major determinants of biomechanical properties of fetal bovine cortical bone. *Bone* 38:300–309
- Viguet-Carrin S, Farlay D, Bala Y, Munoz F, Bouxsein ML, Delmas PD (2008) An in vitro model to test the contribution of advanced glycation end products to bone biomechanical properties. *Bone* 42:139–149
- Shiraki M, Kuroda T, Tanaka S, Saito M, Fukunaga M, Nakamura T (2008) Nonenzymatic collagen cross-links induced by glyco-oxidation (pentosidine) predicts vertebral fractures. *J Bone Miner Metab* 26:93–100
- Schwartz AV, Garnero P, Hillier TA, Sellmeyer DE, Strotmeyer ES, Feingold KR, Resnick HE, Tylavsky FA, Black DM, Cummings SR, Harris TB, Bauer DC (2009) Pentosidine and increased fracture risk in older adults with type 2 diabetes. *J Clin Endocrinol Metab* 94:2380–2386
- Tahara N, Yamagishi SI, Matsui T, Takeuchi M, Nitta Y, Kodama N, Mizoguchi M, Imaizumi T (2010) Serum levels of advanced glycation end products (AGEs) are independent correlates of insulin resistance in nondiabetic subjects. *Cardiovasc Ther*. doi:10.1111/j.1755-5922.2010.00177.x
- Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL, Thorpe SR, Baynes JW, Gans RO, Smit AJ (2004) Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 47:1324–1330
- Fujiwara S, Sone T, Yamazaki K, Yoshimura N, Nakatsuka K, Masunari N, Fujita S, Kushida K, Fukunaga M (2005) Heel bone ultrasound predicts non-spine fracture in Japanese men and women. *Osteoporos Int* 16:2107–2112
- Guo H, Niu K, Monma H, Kobayashi Y, Guan L, Sato M, Minamishima D, Nagatomi R (2010) Association of Japanese dietary pattern with serum adiponectin concentration in Japanese adult men. *Nutr Metab Cardiovasc Dis*. doi:10.1016/j.jnu-mecd.2010.06.006
- Momma H, Niu K, Kobayashi Y, Guan L, Sato M, Guo H, Chujo M, Otomo A, Yufei C, Tadaura H, Saito T, Mori T, Miyata T, Nagatomi R (2010) Skin advanced glycation end product accumulation and muscle strength among adult men. *Eur J Appl Physiol* 111(7):1545–1552
- Noordzij MJ, Lefrandt JD, Graaff R, Smit AJ (2011) Dermal factors influencing measurement of skin autofluorescence. *Diabetes Technol Ther* 13:165–170
- Na R, Stender IM, Henriksen M, Wulf HC (2001) Autofluorescence of human skin is age-related after correction for skin pigmentation and redness. *J Invest Dermatol* 116:536–540
- Fukuda K, Kobayashi S (1973) A study on a self-rating depression scale (author's transl). *Seishin Shinkeigaku Zasshi* 75:673–679 (in Japanese)
- Fountoulakis KN, Lacovides A, Samolis S, Kleanthous S, Kaprinis SG, St Kaprinis G, Bech P (2001) Reliability, validity and psychometric properties of the Greek translation of the Zung Depression Rating Scale. *BMC Psychiatry* 1:6
- Craig CL, Marshall AL, Sjoström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P (2003) International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35:1381–1395
- Sasaki S (2005) Serum biomarker-based validation of a brief-type self-administered diet history questionnaire for Japanese subjects. A research for assessment of nutrition and dietary habit in "Kenko Nippon 21". The Study Group of Ministry of Health, Labor and Welfare of Japan, Tokyo, Japan, pp 10–42 (in Japanese)
- Matsuzawa Y (2005) Metabolic syndrome—definition and diagnostic criteria in Japan. *J Atheroscler Thromb* 12:301
- Gineyts E, Munoz F, Bertholon C, Sornay-Rendu E, Chapurlat R (2010) Urinary levels of pentosidine and the risk of fracture in postmenopausal women: the OFELY study. *Osteoporos Int* 21:243–250
- Loscalzo J (1996) The oxidant stress of hyperhomocyst(e)inemia. *J Clin Invest* 98:5–7
- Shiraki M, Urano T, Kuroda T, Saito M, Tanaka S, Miyao-Koshizuka M, Inoue S (2008) The synergistic effect of bone mineral density and methylenetetrahydrofolate reductase (MTHFR) polymorphism (C677T) on fractures. *J Bone Miner Metab* 26:595–602
- Saito M, Marumo K (2010) Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporos Int* 21:195–214
- Saito M, Marumo K, Soshi S, Kida Y, Ushiku C, Shinohara A (2010) Raloxifene ameliorates detrimental enzymatic and nonenzymatic collagen cross-links and bone strength in rabbits with hyperhomocysteinemia. *Osteoporos Int* 21:655–666
- Goldberg T, Cai W, Peppas M, Dardaine V, Baliga BS, Uribarri J, Vlassara H (2004) Advanced glycooxidation end products in commonly consumed foods. *J Am Diet Assoc* 104:1287–1291
- Gerrits EG, Lutgers HL, Kleefstra N, Graaff R, Groenier KH, Smit AJ, Gans RO, Bilo HJ (2008) Skin autofluorescence: a tool

- to identify type 2 diabetic patients at risk for developing microvascular complications. *Diabetes Care* 31:517–521
34. Lutgers HL, Gerrits EG, Graaff R, Links TP, Sluiter WJ, Gans RO, Bilo HJ, Smit AJ (2009) Skin autofluorescence provides additional information to the UK Prospective Diabetes Study (UKPDS) risk score for the estimation of cardiovascular prognosis in type 2 diabetes mellitus. *Diabetologia* 52:789–797
 35. Sell DR, Monnier VM (1990) End-stage renal disease and diabetes catalyze the formation of a pentose-derived crosslink from aging human collagen. *J Clin Invest* 85:380–384
 36. Sell DR, Monnier VM (1989) Structure elucidation of a senescence cross-link from human extracellular matrix. Implication of pentoses in the aging process. *J Biol Chem* 264:21597–21602
 37. Njeh CF, Hans D, Li J, Fan B, Fuerst T, He YQ, Tsuda-Futami E, Lu Y, Wu CY, Genant HK (2000) Comparison of six calcaneal quantitative ultrasound devices: precision and hip fracture discrimination. *Osteoporos Int* 11:1051–1062
 38. Tang SY, Vashishth D (2010) Non-enzymatic glycation alters micro-damage formation in human cancellous bone. *Bone* 46:148–154
 39. Sanguineti R, Storace D, Monacelli F, Federici A, Odetti P (2008) Pentosidine effects on human osteoblasts in vitro. *Ann N Y Acad Sci* 1126:166–172
 40. Ding KH, Wang ZZ, Hamrick MW, Deng ZB, Zhou L, Kang B, Yan SL, She JX, Stern DM, Isaacs CM, Mi QS (2006) Disordered osteoclast formation in RAGE-deficient mouse establishes an essential role for RAGE in diabetes related bone loss. *Biochem Biophys Res Commun* 340:1091–1097
 41. Miyata T, Notoya K, Yoshida K, Horie K, Maeda K, Kurokawa K, Taketomi S (1997) Advanced glycation end products enhance osteoclast-induced bone resorption in cultured mouse unfractionated bone cells and in rats implanted subcutaneously with devitalized bone particles. *J Am Soc Nephrol* 8:260–270
 42. Valcourt U, Merle B, Gineyts E, Viguet-Carrin S, Delmas PD, Garnero P (2007) Non-enzymatic glycation of bone collagen modifies osteoclastic activity and differentiation. *J Biol Chem* 282:5691–5703
 43. Kume S, Kato S, Yamagishi S, Inagaki Y, Ueda S, Arima N, Okawa T, Kojiro M, Nagata K (2005) Advanced glycation end-products attenuate human mesenchymal stem cells and prevent cognate differentiation into adipose tissue, cartilage, and bone. *J Bone Miner Res* 20:1647–1658
 44. Jin LH, Chang SJ, Koh SB, Kim KS, Lee TY, Ryu SY, Song JS, Park JK (2011) Association between alcohol consumption and bone strength in Korean adults: the Korean Genomic Rural Cohort Study. *Metabolism* 60:351–358