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Bone turnover biomarkers and risk of osteoporotic hip fracture in an Asian population

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Abstract

While epidemiologic studies suggest that bone turnover biomarkers may predict hip fracture risk, findings are inconsistent and Asian data are lacking. We conducted a matched case-control (1:1) study nested in the Singapore Chinese Health Study, a population-based prospective cohort of Chinese men and women (45–74 years) recruited from 1993–1998 in Singapore. One hundred cases with incident hip fracture and 100 individually matched controls were randomly selected from 63,257 participants. Serum bone turnover biomarkers, namely bone alkaline phosphatase (bone ALP), osteocalcin (OC), procollagen type I N propeptide (PINP), N-terminal and C-terminal crosslinking telopeptide of type I collagen (NTX-I and CTX-I) were measured using immunoassays. Hip fracture cases had significantly higher serum levels of OC, PINP, CTX-I and NTX-I than controls ($p<0.05$). There was a dose-dependent positive relationship between OC, PINP, CTX-I and NTX-I and risk of hip fracture (all Ps for trend$\leq$0.006), where the risk was significantly increased by 4.32–8.23 folds for the respective BTM [Quartile (Q) 4 vs. Q1]. The odds ratio [OR (95% CI)] at the highest quartile (Q4) was 6.63 (2.02–21.18) for PINP and 4.92 (1.67–14.51) for CTX-I. The joint effect of PINP and CTX-I showed a 7-fold increase in risk (OR: 7.36; 95% CI: 2.53–21.41) comparing participants with higher levels of PINP (Q4) and CTX-I (Q3-Q4) to those with low levels of PINP (Q1-Q3) and CTX-I (Q1-Q2). Our data demonstrated that higher serum levels of bone turnover biomarkers were associated with increased risk of hip fracture in an Asian population.

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Keywords

bone turnover biomarker; hip fracture; Asian

Introduction

Bone turnover biomarkers (BTMs) reflect bone formation and resorption, and therefore inform the status of bone remodeling, which is a mechanism underlying osteoporosis (1). BTMs are increased during aging in both men and women, and have been suggested to be independent risk factors for osteoporotic fractures (2, 3). According to a World Health Organization report, the majority of osteoporotic fracture patients had bone mineral density (BMD) above the diagnostic criteria (i.e. T score > −2.5) (4), indicating the inadequacy of using BMD alone to identify those at risk of fractures. Thus, the measurement of BTM may serve either as an independent diagnostic and prognostic index or as a complementary indicator to BMD for osteoporotic fractures (5). In fact, recently, a joint effort by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have recommended using BTMs as promising surrogate markers for the prediction for fracture risk independent of BMD (6). In addition, individuals with type 2 diabetes were reported to have higher BMD despite elevated risk of fractures (7, 8). Thus, BTM can potentially be a more sensitive surrogate marker than BMD in assessing fracture risk among patients with diabetes, due to a possible linkage between BTMs and glucose metabolism (9, 10).

BTMs include both bone formation and resorption markers. Bone formation biomarkers are synthesized by osteoblasts and therefore reflect specific osteoblastic functions, and these include bone alkaline phosphatase (bone ALP) (11), osteocalcin (OC) (12), and procollagen type I N propeptide (PINP). These bone formation markers have been proposed as possible predictors for osteoporosis (13–16) and hip fracture risk (17, 18). Bone resorption markers are degradation products of type I collagen (19) and include C-terminal crosslinking telopeptide of type I collagen(CTX-I) and N-terminal crosslinking telopeptide of type I collagen (NTX-I) (19). Increased CTX-I and NTX-I levels have also been reported to be inversely correlated with BMD among women (13, 15) and to be significant predictors for non-spine fractures independent of BMD in several longitudinal studies (13, 20, 21). However, the results from the existing evidence are inconsistent and primarily among Caucasian populations (6).

The objective of this study was to examine the relationship between BTMs and risk of osteoporotic hip fractures in middle-aged to elderly Chinese in Singapore. We hypothesized that higher levels of BTMs might be associated with increased risk of hip fracture.

Materials and Methods

Study population

The current case-control study was nested in the Singapore Chinese Health Study, a population-based prospective cohort to investigate diet, lifestyle factors and risk of chronic

diseases (22). We enrolled 63,257 men (n=27,959) and women (n=35,298) aged 45–74 years between April 1993 and December 1998. The study participants were restricted to two major dialect groups in Singapore, which are the Hokkiens and the Cantonese who originated from Fujian and Guangdong provinces in Southern China, respectively. During the enrollment period, all of our study participants were residents of the government housing estates, which is a government housing facility to accommodate majority (> 80%) of the resident population in Singapore (23). During the recruitment period, 86% of the Singapore population lived in such public housing estates. This study was approved by the Institutional Review Board at the National University of Singapore, and all enrolled participants gave written informed consent.

Baseline assessment was conducted through a face-to-face interview during the initial enrollment. Information was recorded by a trained interviewer using a structured questionnaire, which included demographics, medical history, cigarette smoking, alcohol consumption, physical activity, and detailed menstrual and reproductive history (women only). Habitual dietary intake for each participant was recorded using a validated 165-item semi-quantitative food frequency questionnaire, which incorporated common and distinct food items in Singapore (22).

During April 1994 to December 1999, a random 3% of the study participants donated blood and single-void urine specimens for research. Between January 2000 and April 2005, we extended the biospecimen collection to 32,543 participants, which represented a consent rate of about 60% of surviving cohort participants at that time. For each subject, there was only one time-point collection of biospecimen in either collection wave. Correspondingly, there were 4 case-control sets (n=8) from the period of 1994–1999, constituting 4% of this study, and 96 case-control sets (n=192) from the period of 2000–2005. All blood samples were taken between 8 am and 12 pm. Fasting was defined as blood drawn at least 8 hours after the last intake of food and drinks. There were 21.5% subjects (n=43) who were fasted when blood was drawn. After collection, the biospecimens were immediately placed on ice during transport to the laboratory and processed within 6 hours. They were subsequently stored in liquid nitrogen tanks at −180°C until year 2001, when they were moved to freezers at −80°C for long-term storage. All blood collected after year 2001 was stored in freezers at −80°C immediately (24).

Cases and controls selection

Hip fracture cases were identified via record linkage with hospital discharge database of the MediClaim System, which captures inpatient discharge information from all public and private hospitals in Singapore. All cases were verified by surgical and/or medical records. Only men and women without prior history of hip fracture at recruitment were eligible for the present study.

Among the 1,630 hip fracture incident cases in the cohort, we identified 127 cases of men and 138 cases of women who had donated blood prior to hip fractures. We estimated that with a sample size of 100 cases and 100 matched controls, assuming a Type I error probability associated with the null hypothesis at 0.05, our study had an approximately 80% power to detect a statistically significant odds ratio of 3.0 for the highest versus the lowest

quartile of the respective BTM. We then randomly chose 50 hip fracture cases for each gender by simple random sampling and found a matching control for each case with the following matching criteria: age at study enrollment (±3 years), dialect group (Hokkien, Cantonese), date of study enrollment (±2 year), and date of biospecimen collection (±6 months). The selected controls must not have any fracture at the time of hip fracture of their index cases. Compared with the hip fracture patients who were not included in this study (n=1,530), the 100 randomly chosen cases were similar in age at hip fracture, dialect group and body mass index (BMI) at baseline ($p>0.05$). The average age of incident hip fracture was 75.1 [standard deviation (SD) 6.3] years for the 100 cases included in this study and 74.4 (SD 7.5) years for the 1,530 cases not included in this study.

Bone turnover biomarkers (BTMs)

Serum samples of a given matched set (containing the samples from the case and his/her matched control) were arranged in a random order, identified only by unique codes, and tested by the Clinical and Translational Science Award (CTSA) Immunochemical Core Laboratory at the Mayo Clinic in Rochester, Minnesota, U.S.A. The laboratory personnel were blinded to the status of the case and control samples. Serum samples were thawed at 4°C and individually filtered via 0.22 μm sterile cartridges. Filtered sera were collected in aliquots for measurements of five BTMs, bone ALP, OC, PINP, CTX-I and NTX-I. Bone ALP was measured using an immunoassay (the assay has a 3–8 % cross-reactivity with liver alkaline phosphatase), and its enzymatic activity was measured at intra- and inter-assay coefficients of variation (CV) < 10% at limit of detection (LOD) of 18 U/L and < 4% at LOD of 16.5 U/L, respectively; Metra Biosystems, Mountainview, CA (25). OC was measured by a 2-site immunenozymatic sandwich assay on the Roche Cobas e411 (intra- and inter- assay CV <3% at LOD of 1.62ng/mL and 3.9% at LOD of 15.8 ng/mL, respectively; Roche Diagnostics, Indianapolis, IN) (26). PINP was measured by a double antibody (intra- and inter-assay CV was 2.3% at LOD of 44.5 μg/L and 3.8% at LOD of 28.0 μg/L, respectively; Orion Diagnostica, Espoo, Finland: distributed by Diasorin, Stillwater, MN) (27). CTX-I was measured by a two-site immunoenzymatic sandwich assay on the Roche Cobas e411 (intra- and inter- assay CV < 7.8% at LOD of 0.046 ng/mL and <8.5% at LOD of 0.291 ng/mL, respectively; Roche Diagnostics, Indianapolis, IN) (28). NTX-I was measured by a quantitative competitive-inhibition enzyme-linked immunosorbent assay (intra- and inter- assay CV was <6.9% at LOD of 7.5 nmol BCE/L and 7.1% at 26.5 nmol BCE/L, respectively; Ostex International, Seattle, WA) (29).

Statistical analysis

Student’s t-test (for normally distributed continuous variables) and Chi-square test (for categorical variables) were used to compare baseline characteristics between hip fracture cases and controls. The distributions of all biomarkers measured were markedly skewed with a long tail toward high values, which were corrected, to a large extent, by transforming the original values to logarithmic values. Therefore, formal statistical test was performed on logarithmically transformed values, and geometric means were presented. The analysis of covariance (ANCOVA) was used to examine the differences in the concentrations of serum biomarkers between hip fracture cases and controls in all subjects, in men and women separately, and between those with and without baseline history of diabetes.

We applied conditional logistic regression to estimate relative risk of hip fracture associated with higher levels (2^{nd} to 4^{th} quartiles or Q2 to Q4) of each BTM compared to the lowest quartile (Q1) for all subjects and in separate analysis for men and women. The quartile cut-off values were based on the BTM's distributions among all controls or controls within each gender (for gender-specific analysis only). Model 1 included the matching variables only: age, sex, dialect group, date of study enrollment, and date of biospecimen collection. In addition to variables included in Model 1, Model 2 included BMI (kg/m^2, continuous), level of education (no formal education, primary school, secondary school or higher), smoking status (never smokers, ex-smokers, current smokers), physical activity (none, 0.5–3.9 hrs weekly, ≥4 hrs weekly), and dietary soy isoflavones (quartiles, mg/1,000 kcal/day), pyridoxine (quartiles, mg/1,000kcal/day), and β-carotene (quartiles, μg/1,000kcal/day); all of these covariates were associated with risk of hip fracture in our previous analyses (30–32). In addition to the variables in Model 2, Model 3 included a variable for self-reported history of physician-diagnosed diabetes mellitus (yes, no), which has been shown to significantly increase risk of hip fracture in our study population (33). Furthermore, the increase in hip fracture risk per SD increase (SD defined among controls only) in each BTM was also calculated for all subjects and for men and women separately. The magnitude of the association was assessed by odds ratio (OR) and the corresponding 95% confidence interval (CI). To examine linear trend, ordinal values of the quartile of each BTM was entered as a continuous variable in the logistic regression model.

All statistical analysis was conducted using SAS Version 9.2 (SAS Institute, Inc., Cary, North Carolina). All reported p values were two-sided, and $p < 0.05$ was considered statistically significant.

Results

Among the 100 cases of hip fracture, the mean time interval from blood draw to the occurrence of hip fracture was 5.0 (SD 2.6) years. The mean age of patients at the occurrence of hip fracture was 75.1 (SD 7.6) years. Baseline characteristics between the hip fracture cases and controls are presented in Table 1. Compared to the non-hip fracture controls, there was no statistically significant differences in BMI, level of education, cigarette smoking (status, smoking density and duration), weekly moderate physical activity, serum 25(OH)D level, dietary intake of calcium, pyridoxine and β-carotene, or history of stroke among the hip fracture cases ($p>0.05$). However, the cases had significantly lower dietary intake of soy isoflavones and higher prevalence of diabetes mellitus than the controls ($p<0.05$). In addition, at blood draw, all women were postmenopausal among the cases and only one woman was premenopausal among the controls.

Table 2 shows the geometric means of all five BTMs in serum samples collected from the cohort participants who subsequently developed hip fracture (cases) and those who remained free of hip fracture (controls) for all subjects, men and women separately. In all subjects, cases had significantly higher serum levels of OC, PINP, CTX-I and NTX-I than controls ($p\leq0.007$). Serum bone ALP levels were higher in cases than controls, but the difference was not statistically significant. In men, statistically significant higher serum levels of OC, PINP, CTX-I and NTX-I were also found in cases than in controls ($p\leq0.04$). However, in women,

although all BTMs were higher in cases compared to controls, only the differences in serum levels of OC and CTX-I reached statistical significance ($p \leq 0.05$). The Spearman correlation coefficients among the five BTMs after adjustment for gender ranged from 0.42 to 0.76; the highest correlation coefficient was 0.76 between CTX-I and NTX-I and 0.75 between OC and PINP. The correlation coefficient was 0.59 between PINP and CTX-I.

The association between serum BTMs and risk of hip fracture is shown in Table 3. There was a statistically significant dose-dependent positive association for OC, PINP, CTX-I and NTX-I with risk of hip fracture. After adjustment for history of diabetes mellitus and other potential confounders, ORs (95% CIs) of hip fracture for the highest quartile of OC, PINP, CTX- I, and NTX-I were 8.23 (2.26–30.03), 6.63 (2.02–21.81), 4.92 (1.67–14.51) and 4.32 (1.39–13.45), respectively, compared to their respective lowest quartile (Q1) (all p for trend ≤ 0.006). There was no statistically significant association between serum bone ALP and hip fracture risk. Similarly, the ORs for hip fracture risk per SD increase for OC, PINP, CTX-I and NTX-I were statistically significant and ranged from 1.55 to 1.95 (Table 3). Separate analyses among men and women yielded generally the same conclusion as the results for both genders combined, although some of the risk estimates did not reach statistical significance due to a small sample size. The results between men and women were also comparable (Supplemental Table 1 and 2).

To determine which individual BTM was independent risk factor, a stepwise selection approach with conditional logistic regression was employed with a variable entry p-value of 0.30 and a retention p-value of 0.35. The stepwise approach yielded PINP and CTX-I as the final two independent variables in the most parsimonious model. We further assessed the joint effect of PINP and CTX-I on the risk of hip fracture (Table 4). Currently, there are no biologically or clinically defined values of low or high levels for these two biomarkers. Hence, we used the ORs for the quartile categories of PINP and CTX-I in Table 3 as a guide to define the risk group. Because the increase in risk was statistically significant in the highest quartile compared to the lowest quartile for PINP, we defined the high-level group as Q4 and the low-level group as Q1-Q3 for PINP. For CTX-I, a statistically significant increase in risk was found in the two upper quartiles, and these became the high-level group (Q3-Q4) while the two lower quartiles formed the low-level group (Q1-Q2) for CTX-I. Hence, in examining the joint effects of CTX-I and PINP, the reference group was defined by the participants in the low-level groups for both BTMs, i.e. PINP (Q1-Q3) and CTX-I (Q1-Q2). Compared to the reference group, those who were in the high-level group for either PINP (Q4) or CTX-I (Q3-Q4) had about 3-fold increase in risk of hip fracture. The greatest risk was found among those in the high-level group, i.e. CTX-I (Q3-Q4) and PINP (Q4); these participants had a 7.36-fold increase in hip fracture risk (OR: 7.36; 95% CI: 2.53–21.41) compared to those in the reference group (Table 4). The *P*-value for the multiplicative interaction was not significant (*p* for interaction=0.69). Because only 21.5% of all subjects were fasted during blood draw, further adjusted for fasting status yielded materially the same results (data not shown).

Furthermore, we noted that after controlling for baseline physician-diagnosed history of diabetes, the adjusted OR became substantially higher, suggesting a negative confounding effect by history of diabetes in the association of BTM with risk of hip fracture. Further

analysis showed that this was because among both cases and controls, subjects with diabetes at baseline (n=20) had lower levels of BTMs, particularly OC, PINP and NTX-I, as compared to those without history of diabetes (Table 5).

Discussion

To our best of knowledge, this is the first longitudinal study to prospectively assess risk of hip fracture using a panel of five BTMs and confirmed PINP and CTX-I as two best predictors for hip fracture in an Asian population. Our findings showed higher serum levels of OC, PINP, CTX-I and NTX-I in pre-fracture blood samples of the hip fracture cases as compared to the non-fracture controls, and these BTMs were in turn positively associated with risk of hip fracture. Furthermore, our results confirmed that serum PINP and CTX-I were independent predictors for hip fractures among the BTMs we examined in this study. This is consistent with the two recommended analytes by the IOF and the IFCC (6).

Findings from the present study are generally consistent with those from the previous studies among Caucasian populations. Several prospective studies have shown that one or two of the bone formation (OC, PINP) and/or resorption (CTX-I) markers in the serum were significantly associated with increased risk of hip fracture among Caucasian populations (17, 18, 20, 34, 35). Among these studies, only one study was conducted among elderly men (34). A case-cohort study nested in the Osteoporotic Fractures in Men (MrOS) cohort of men aged over 65 years showed that, after a follow-up of 5 years, a higher level of serum PINP or CTX-I was related to bone loss and risk of hip fracture [relative hazards (RH) = 2.13 (95% CI: 1.23–2.68) and RH=1.76 (95% CI: 1.04–2.98), respectively], comparing the highest quartile to the lower three quartiles. However, further adjusting for BMD and other clinical risk factors attenuated the associations and the statistical significance was lost (34). In a case-control study nested in the EPIDOS prospective study of healthy French women aged 75 years or older, OC was associated with increased risk of hip fracture by nearly 2 folds (OR: 1.8, 95% CI: 1.0–3.0) independent of femoral neck bone density after a follow-up of 22 months (18). Other prospective cohort studies among women also suggested that a higher bone ALP, CTX-I or NTX-I level was associated with greater risk of all osteoporotic fractures (21, 36–39).

Compared to these studies among Caucasian populations, our findings further demonstrated that multiple bone formation and resorption markers may significantly increase risk of hip fracture among middle-aged to elderly Chinese. Compared to the reference serum levels of BTMs reported among healthy Caucasian men and/or women, the bone ALP level of the controls in the present study [median (interquartile): 37.80 (29.4–48.3) U/L] tended to be higher than that in these studies (40–42), while the levels of OC [18.1 (13.8–24.1) ng/mL], PINP [44.5 (34.2–55.0) μg/L] and CTX-I [0.28 (0.21– 0.41) ng/mL] were within the reference range specified in these studies (40, 42). However, it should be noted that the assays for BTM measurement were not standardized and may be different among studies (40, 42). Furthermore, a number of factors, such as circadian, fasting status, lifestyle factors and health condition can also affect the variability of each BTM (6).

In line with the recommended analytes by the IOF and IFCC, our data confirmed that PINP and CTX-I were the best risk predictors for hip fractures among the five BTMs we examined. In addition, this study is the first to show additive effect by combining PINP and CTX-I in hip fracture risk, and this was supported by a recent meta-analysis which reported both serum PINP and CTX-I significantly increased risk of fracture (43). Hence, our finding has provided additional evidence for the utility of PINP and CTX-I as a panel of standardized BTMs for fracture prediction.

More interestingly, in the present study, individuals with a history of diabetes mellitus had lower levels of BTMs, particularly OC, PINP and NTX-I ($P<0.05$) than those without a history of diabetes in both cases and controls. This was supported by several studies that reported lower levels of OC (8, 10, 44–46) and CTX-I (8) in diabetic patients as compared to non-diabetic individuals in different populations. While most of these studies were conducted in Caucasian populations, there were two relevant Asian studies. A cross-sectional study in Chinese postmenopausal women reported lower levels of both OC and cortical BMD in the tibia of women with diabetes than those without (47). The other case-control study in Japan reported lower levels of OC, PINP and CTX-I in both men and women with diabetes than their counterparts without diabetes (45). Taken together, these findings suggest a lower cut-off value of BTMs may be required for the identification of individuals with type 2 diabetes at high risk of hip fractures.

There are several proposed mechanisms that higher bone turnover may be associated with greater fracture risk. First, bone turnover plays a central role directly and indirectly in the mechanical resistance of the skeleton (48). Bone turnover may also have direct impacts in bone mineral density, bone macro- and micro- architecture and bone matrix, all of which are factors involved in the development of osteoporotic fractures (1, 49). Another speculation is that despite higher bone turnover, the newly formed bones are less well mineralized than the mature bones (50, 51), which may be due to the lack of enzymatic cross-linking for calcium deposition in the collagen matrix in osteoporosis (51). In addition, although the clear biological mechanisms between diabetes and bone changes and deterioration remain to be elucidated, several possible interlinking among insulin, receptor activator of nuclear factor kappa-B ligand (RANKL) pathway, Wnt/beta-catenin pathway, alteration of collagen crosslinking, and BTM changes have been proposed (7). Additionally, more data is needed to assess the biologically sensitive surrogate markers for risk of fracture among diabetic individuals.

The strengths of the present study are the presumed lack of recall bias in exposure data from the questionnaire and the collection of blood specimens prior to hip fracture occurrence. In addition, we considered all of the previously reported dietary, lifestyle and health factors in the model, and our results suggest that these factors could confound the association and underestimate the true associations if unaccounted for. One of the limitations of this study is that the results were based on a single time-point collection of blood samples, which may not fully reflect the biomarkers of bone remodeling. However, since the serum samples were collected before fractures, such misclassifications are generally non-differential in nature, and may result in an underestimation of the true effect size of BTM on hip fracture risk. Another limitation is the lack of measurements on BMD, hence we were unable to examine

whether bone turnover biomarkers were independent of BMD in relation to risk of hip fracture.

In conclusion, the present study demonstrated that higher serum levels of OC, PINP, CTX-I and NTX-I were associated with subsequent risk of hip fracture in an Asian population, and confirmed PINP and CTX-I as the best BTM predictors for incident hip fractures. Future studies are warranted to quantify the ideal cut-off values to optimize the sensitivity and specificity of these two BTMs with or without BMD for risk prediction of hip fracture, and to evaluate whether a lower cut-off BTM level is required to identify risk of hip fracture among individuals with diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Serum bone turnover markers (BTMs) are associated with increased hip fracture risk.
- Serum PINP and CTX-I are two independent predictors for incident hip fractures.
- Patients with diabetes may require lower BTM levels to define hip fracture risk.

Table 1

Baseline characteristics [percent or mean (SD)] of individuals who developed hip fracture (cases) and those who remained free of hip fracture (controls), The Singapore Chinese Health Study

Characteristics	Cases	Controls	p-value*
Number of subjects	100	100	…
Age at blood draw, mean (SD)	70.1 (7.4)	69.9 (7.2)	0.91
Body mass index (kg/m2), mean (SD)	22.7 (3.4)	23.4 (3.1)	0.15
Level of education, n (%)			0.85
No formal education	30 (30)	32 (32)	
Primary school	50 (50)	46 (46)	
Secondary school and above	20 (20)	22 (22)	
Current smokers, n (%)	20 (20)	18 (18)	0.72
Moderate activity>0.5 hours per week, n (%)	32 (32)	29 (29)	0.65
Serum 25 (OH)D (ng/mL), geometric mean (95% CI)	24.2 (23.1, 25.4)	23.9 (22.7, 25.1)	0.83
Calcium (mg/day), mean (SD)	419.0 (232.9)	423.4 (207.2)	0.89
Soy isoflavones (mg/1000 kcal/day), mean (SD)	10.4 (7.8)	13.4 (11.4)	0.03
Pyridoxine (mg/1000kcal/day), mean (SD)	0.68 (0.14)	0.71 (0.16)	0.20
β-Carotene (μg/1000 kcal/day), mean (SD)	1,365.4 (769.6)	1,392.6 (783.8)	0.80
History of stroke, n (%)	3 (3)	1 (1)	0.32
History of diabetes mellitus, n (%)	15 (15)	5 (5)	0.02

*Two-sided p-value was derived from *t* test for continuous variables and from chi-squared test for categorical variables.

Table 2

Geometric means (95% confidence intervals) of serum bone turnover biomarkers in individuals who developed hip fracture (cases) and those who remained free of hip fracture (controls), the Singapore Chinese Health Study

	Geometric mean (95% CI)*		
BTM	**Cases**	**Controls**	**p-value**
All subjects	100	100	
Bone ALP (U/L)	39.4 (36.3–42.7)	36.7 (33.9–39.6)	0.10
OC (ng/mL)	19.9 (18.1–21.8)	17.3 (15.8–18.9)	0.006
PINP (μg/L)	48.6 (44.1–53.5)	42.1 (38.3–46.2)	0.007
CTX-I (ng/mL)	0.36 (0.32–0.40)	0.28 (0.24–0.32)	0.0003
NTX-I (BCE/L)	14.0 (13.1–15.0)	12.3 (11.5–13.1)	0.0006
Men	50	50	
Bone ALP (U/L)	37.4 (34.0–41.1)	35.8 (32.8–39.0)	0.47
OC (ng/mL)	19.9 (17.7–22.3)	17.0 (15.3–19.0)	0.04
PINP (μg/L)	44.7 (40.5–49.3)	39.2 (35.8–42.8)	0.04
CTX-I (ng/mL)	0.34 (0.30–0.40)	0.26 (0.24–0.30)	0.007
NTX-I (BCE/L)	13.8 (12.8–15.0)	11.6 (10.8–12.5)	0.001
Women	50	50	
Bone ALP (U/L)	46.7 (37.1–58.8)	42.5 (33.5–53.8)	0.16
OC (ng/mL)	21.9 (17.0–28.0)	18.9 (14.6–24.4)	0.05
PINP (μg/L)	57.2 (41.7–78.4)	48.0 (34.6–66.4)	0.06
CTX-I (ng/mL)	0.42 (0.30–0.60)	0.34 (0.24–0.46)	0.01
NTX-I (BCE/L)	15.8 (13.3–18.7)	14.6 (12.3–17.5)	0.15

*Adjusted for matching factors (age, gender, and dialect group), and BMI (kg/m^2, continuous), level of education (no formal education, primary school, secondary school or higher), smoking status (never smokers, ex-smokers, current smokers), physical activity (none, 0.5–3.9 hrs weekly, ≥4 hrs weekly), soy isoflavones (mg/1,000 kcal/day in quartile), pyridoxine (mg/1,000 kcal/day in quartile), β-carotene (μg/1,000 kcal/day in quartile), and baseline self-reported physician-diagnosed history of diabetes mellitus (yes, no).

Table 3

The association between serum bone turnover biomarkers in quartile and the risk of hip fracture, The Singapore Chinese Health Study

	Odds ratio (95% confidence interval)					
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	**p for trend**	**Per SD[d]**
Bone ALP (U/L), Median (IQR)	26.5 (23.0–27.8)	34.0 (31.7–36.4)	42.2 (39.8–44.0)	54.6 (50.5–61.1)		
Cases/Controls	16/25	27/25	32/25	25/25		
Model 1[a]	1.0 (Referent)	1.68 (0.75–3.80)	2.08 (0.89–4.88)	1.56 (0.66–3.70)	0.28	1.20 (0.89–1.62)
Model 2[b]	1.0 (Referent)	2.29 (0.91–5.77)	2.74 (1.03–7.26)	2.35 (0.84–6.57)	0.11	1.32 (0.93–1.87)
Model 3[c]	1.0 (Referent)	2.32 (0.9–5.94)	2.93 (1.07–8.01)	2.41 (0.84–6.95)	0.10	1.40 (0.95–2.07)
OC (ng/mL), median (IQR)	11.3 (10.0–12.5)	16.2 (15.1–16.8)	21.1 (18.9–23.0)	28.9 (26.7–33.3)		
Cases/Controls	12/25	27/25	26/25	35/25		
Model 1[a]	1.0 (Referent)	2.10 (0.89–4.97)	2.32 (0.90–5.97)	3.22 (1.26–8.22)	0.02	1.32 (0.93–1.87)
Model 2[b]	1.0 (Referent)	1.84 (0.72–4.68)	2.48 (0.88–7)	3.38 (1.2–9.47)	0.02	1.32 (0.92–1.90)
Model 3[c]	1.0 (Referent)	2.86 (0.92–8.90)	6.29 (1.68–23.52)	8.23 (2.26–30.03)	0.001	1.55 (1.04–2.31)
PINP (μg/L), median (IQR)	26.8 (23.6–30.9)	39.8 (37.1–42.7)	49.3 (46.8–52.7)	67.9 (59.0–82.6)		
Cases/Controls	16/25	25/25	20/26	39/24		
Model 1[a]	1.0 (Referent)	1.89 (0.72–4.97)	1.52 (0.58–3.95)	3.39 (1.27–9.07)	0.03	1.35 (0.97–1.88)
Model 2[b]	1.0 (Referent)	2.30 (0.80–6.60)	1.50 (0.50–4.47)	4.54 (1.49–13.77)	0.02	1.45 (1.02–2.06)
Model 3[c]	1.0 (Referent)	2.94 (0.97–8.92)	2.09 (0.65–6.69)	6.63 (2.02–21.81)	0.004	1.62 (1.10–2.37)
CTX-I (ng/mL), median (IQR)	0.18 (0.13–0.20)	0.25 (0.23–0.27)	0.34 (0.31–0.37)	0.51 (0.47–0.66)		
Cases/Controls	13/25	21/25	33/25	33/25		
Model 1[a]	1.0 (Referent)	1.54 (0.68–3.48)	2.63 (1.09–6.37)	2.68 (1.09– 6.57)	0.02	1.43 (1.06–1.94)
Model 2[b]	1.0 (Referent)	1.53 (0.60–3.91)	3.49 (1.26–9.68)	4.00 (1.43–11.18)	0.004	1.58 (1.14–2.19)
Model 3[c]	1.0 (Referent)	1.39 (0.54–3.59)	3.66 (1.28–10.46)	4.92 (1.67–14.51)	0.002	1.78 (1.24–2.56)
NTX-I (BCE/L), median (IQR)	9.1(7.9–10.2)	12.2 (11.60–12.60)	14.4 (13.80–15.20)	18.6 (16.90–20.70)		
Cases/Controls	21/25	21/25	20/25	38/25		
Model 1[a]	1.0 (Referent)	1.15 (0.49– 2.71)	1.08 (0.42–2.79)	1.93 (0.81–4.61)	0.10	1.42 (1.04–1.93)
Model 2[b]	1.0 (Referent)	1.33 (0.50–3.52)	1.14 (0.37–3.45)	2.62 (0.93–7.35)	0.04	1.53 (1.08–2.16)

	Odds ratio (95% confidence interval)					
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	**p for trend**	**Per SD[d]**
Model 3[c]	1.0 (Referent)	1.57 (0.56–4.42)	1.70 (0.50–5.73)	4.32 (1.39–13.45)	0.006	1.95 (1.29–2.93)

[a] Model 1: conditional logistic regression model that adjusted for matching factors including age at study enrollment (±3 years), sex, dialect group (Hokkien, Cantonese), date of study enrollment (±2 year), and date of biospecimen collection (±6 months);

[b] Model 2 – further adjusted for BMI (kg/m^2, continuous), level of education (no formal education, primary school, secondary school or higher), smoking status (never smokers, ex-smokers, current smokers), physical activity (none, 0.5–3.9 hrs weekly, ≥ 4 hrs weekly), soy isoflavones (mg/1,000 kcal/day in quartile), pyridoxine (mg/1,000 kcal/day in quartile), β-carotene (μg/1,000 kcal/day in quartile;

[c] Model 3 – further adjusted for self-reported history of physician-diagnosed diabetes mellitus (yes, no) at baseline;

[d] per SD increase in biomarker levels.

Table 4

Joint effect of serum PINP (μg/L) and CTX-I (ng/mL) on the risk of hip fracture, the Singapore Chinese Health Study

	PINP (Q1-3)		PINP (Q4)	
	Cases/Controls	**OR* (95% CI)**	**Cases/Controls**	**OR* (95% CI)**
CTX-I (Q1-2)	26/43	Reference	8/7	3.42 (0.63–18.52)
CTX-I (Q3-4)	35/33	3.17 (1.26–7.99)	31/17	7.36 (2.53–21.41)

*Adjusted for BMI (kg/m^2, continuous), level of education (no formal education, primary school, secondary school or higher), smoking status (never smokers, ex-smokers, current smokers), physical activity (none, 0.5–3.9 hrs weekly, ≥ 4 hrs weekly), soy isoflavones (mg/1,000 kcal/day in quartile), pyridoxine (mg/1,000 kcal/day in quartile), β-carotene (μg/1,000 kcal/day in quartile) and baseline self-reported physician-diagnosed history of diabetes mellitus (yes, no).

Table 5

Comparison of bone turnover biomarkers in participants with and without a history of physician-diagnosed diabetes at baseline, the Singapore Chinese Health Study

Bone turnover biomarkers	Geometric mean* (95% CI)		
Both cases and controls	**Subjects with diabetes**	**Subjects without diabetes**	**p-value**
Number of subjects	20	180	
Bone ALP (U/L)	37.48 (32.32–43.48)	38.08 (35.82–40.48)	0.84
OC (ng/mL)	13.44 (11.26–16.06)	19.08 (17.74–20.54)	0.0002
PINP (μg/L)	35.18 (29.18–42.42)	46.82 (43.34–50.58)	0.0035
CTX-I (ng/mL)	0.26 (0.20–0.34)	0.32 (0.28–0.34)	0.11
NTX-I (BCE/L)	11.62 (10.12–13.36)	13.62 (12.86–14.42)	0.03
Controls only			
Number of subjects	5	95	
Bone ALP (U/L)	31.04 (23.2–41.54)	36.82 (33.98–39.92)	0.26
OC (ng/mL)	12.62 (8.62–18.46)	17.90 (16.12–19.88)	0.08
PINP (μg/L)	35.14 (23.72–52.06)	43.34 (38.88–48.32)	0.31
CTX-I (ng/mL)	0.20 (0.12–0.32)	0.28 (0.26–0.32)	0.14
NTX-I (BCE/L)	10.32 (7.76–13.72)	12.74 (11.78–13.8)	0.16
Cases only			
Number of subjects	15	85	
Bone ALP (U/L)	39.58 (33.02–47.46)	39.30 (35.76–43.18)	0.94
OC (ng/mL)	14.00 (11.44–17.1)	20.38 (18.36–22.62)	0.0004
PINP (μg/L)	35.72 (28.74–44.4)	50.40 (45.02–56.42)	0.003
CTX-I (ng/mL)	0.30 (0.22–0.40)	0.34 (0.30–0.40)	0.28
NTX-I (BCE/L)	12.86 (10.92–15.14)	14.38 (13.22–15.66)	0.19

*Adjusted for hip fracture status (yes, no, for overall only), age (years, continuous), gender (men, women), BMI (kg/m^2, continuous), level of education (no formal education, primary school, secondary school or higher), smoking status (never smokers, ex-smokers, current smokers), physical activity (none, 0.5–3.9 hrs weekly, ≥ 4 hrs weekly), soy isoflavones (mg/1,000 kcal/day in quartile), pyridoxine (mg/1,000 kcal/day in quartile) and β-carotene (μg/1,000 kcal/day in quartile).