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RESEARCH ARTICLE

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# Evaluation of bone metabolism-associated biomarkers in Tibet, China

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## Abstract

**Aim:** To measure and evaluate the distribution and possible contributing factors of seven bone metabolism-associated biomarkers in Tibet, a plateau province of China.

**Methods:** A total of 1615 individuals were recruited from Tibet at three different altitudes. The levels and possible contributing factors of serum calcium, serum phosphorus, ALP, 25OHD, PINP, CTX, and PTH were evaluated.

**Results:** In total, 1246 Tibetan adults (males: $n = 543$) were eventually enrolled in this study. Multiple linear regression recognized age, sex, altitude, and BMI as the major effect factors. The levels of ALP, PINP, and CTX in males continuously decreased with age; however, those in females increased after approximately 39 years of age. Males had higher 25OHD levels (23.9 vs. 15.4 ng/ml) but lower levels of serum phosphorus (1.12 vs. 1.19 mmol/L) and PTH (41.3 vs. 47.4 pg/ml) than females. Before the age of 50, males had higher levels of calcium, ALP, PINP, and CTX than females, and the opposite trend was observed after the age of 50. The highest levels of serum calcium and phosphorus and the lowest levels of PINP and CTX were found in the Shigatse/Lhasa region, suggesting a better bone metabolism status. Compared with reports from plain areas of China, significantly higher levels of PINP (65.3 vs. 49.36 ng/ml) and CTX (0.46 vs. 0.37 ng/ml) were recorded in Tibetan adults.

**Conclusion:** A more active bone turnover status was found in Tibetan adults than in individuals from the plain areas of China.

KEYWORDS
biochemical markers, bone metabolism, plateau, Tibetan

## 1 | INTRODUCTION

With the aging of the global population, osteoporosis is now the seventh most common disease threatening human health, and up to 60% of the elderly in China suffer from it.[1,2] Osteoporosis can lead to serious complications such as fracture, which is estimated to increase to 5.99 million by 2050 in China.[3] Even in healthy adults, bone mass decreases at an approximate rate of 0.5% per year.[4] However, many patients at an increased risk of bone fracture do not undergo timely diagnosis and appropriate treatment for osteoporosis.[5] Therefore, it is crucial to evaluate and monitor the status of human bone metabolism regularly and effectively.

Dual-energy X-ray absorptiometry remains the gold standard method for the diagnosis of osteoporosis.[6] However, the measurement of bone mineral density (BMD) can indicate only the static bone status during a time period, which is not conducive to the early diagnosis of related diseases. Recently, bone metabolism-associated biochemical markers, including general biochemical markers such as calcium and phosphorus in both serum and urine, bone metabolism regulatory hormones such as parathyroid hormone (PTH), vitamin D, and their metabolites, along with bone turnover markers such as serum alkaline phosphatase (ALP), N-terminal pro-peptide of type I procollagen (PINP), and bone resorption markers such as C-terminal telopeptide of type I collagen (CTX), were recognized as good indices for the diagnosis and monitoring of bone-related diseases; these markers can rapidly indicate the status of human bone loss or formation and predict the risk of bone fracture.[7,8]

Previous studies found that extreme altitude was closely related to bone-vascular coupling and bone formation, in which hypoxia-inducible factors played a critical role.[9] Additionally, erythropoietin, which is increased under hypoxic stress, could negatively regulate bone mass with significantly increased bone resorption.[10] When oxygen partial pressure in arterial blood <40 mmHg, a sustained hypoxic environment could negatively influence the bone mass and bone quality; however, hypoxic conditioning could also be a potential nonpharmacological strategy to treat skeletal diseases.[9,11] These different effects could be related to the discrepancies in oxygen concentration and exposure time, and the specific effect on the process of bone metabolism and its potential mechanisms remain unclear. The Tibet Autonomous Region is a plateau province with an average altitude of more than 4000 m and is the major area of the distribution of the Tibetan population in China. A limited number of studies have evaluated the BMD values of Tibetan individuals in Tibet and found a significant difference from the plain area.[12–14] However, more extensive research, especially multicenter studies aimed at evaluating the distribution of bone metabolism-associated biomarkers in Tibet, is still lacking, not to mention the establishment of specific reference intervals (RIs), which could affect timely and appropriate clinical diagnosis and treatment.

In this study, we comprehensively evaluated the levels of bone metabolism-associated biomarkers in Tibetan adults at three different altitudes and determined the important contributing factors.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

From 2016 to 2018, based on the cluster sampling method with a standard questionnaire including information on diet, exercise, and night shifts, we continuously and randomly recruited 1615 participants from individuals of the general Tibetan population who had resided in Tibet for more than 1 year. To avoid the effect of seasonal variation, all of the sampling was conducted within July to September. This multicenter study was performed on individuals residing across a wide region in Tibet, which included four cities at three altitude levels: Group A (Ali, altitude: 4298–4352 m), Group B (Shigatse and Lhasa, altitude: 3670–3835 m), and Group C (Nyingchi, altitude: approximately 2900 m). A total of 1246 apparently healthy individuals were eventually enrolled in the study, and the specific exclusion criteria were as follows[15]:

1. Sex and/or age unknown;
2. Aged <19 or >64 years old;
3. Not of Tibetan nationality;
4. Diagnosis of systemic diseases including diabetes, cardiovascular disease, hypertension, kidney disease, autoimmune disease, cancer, and bone metabolism-associated diseases such as osteoporosis and bone pain;
5. Hospitalized within the preceding 6 months;
6. History of illness owing to inflammation, cold, or other reasons in the 4 weeks preceding the period of recruitment.
7. Pregnant, lactating, or had given birth within the past one year
8. Recent intake of hormone drugs.

### 2.2 | Preparation and measurements

In this study, the sample quality was strictly controlled. All of the processes of sample collection, processing, and measurements were well trained and monitored, as shown in a previous study.[15] In brief, all fasting blood or urine samples in the morning were drawn in tubes, centrifuged within 1 h, and divided and stored at −80°C within 4 h. The samples were transported using the cold chain transportation system with strict temperature control to the Department of Laboratory Medicine of the central hospital, where uniform measurement experiments were conducted. Biochemical parameters, including serum calcium, serum phosphorus, ALP, albumin (Alb), alanine transaminase (ALT), triglyceride (TG), and total cholesterol (TC), were measured using an AU 5800 automatic biochemical analyzer (Beckman Coulter), and the levels of calcium, phosphorus, and creatinine (Cr) in urine were measured using a Beckman AU 2700 Automatic Biochemical Analyzer. The levels of serum 25 hydroxyvitamin D (25OHD), PINP, CTX, and PTH were measured using an E601 electrochemiluminescence analyzer (Roche) coupled with the corresponding reagents, calibrators, and quality controls. The clinical reference intervals (RIs) used at the Department of Laboratory

Medicine of the central hospital for serum calcium, serum phosphorus, ALP, PTH, 25OHD, and CTX are shown in Table S1. Since the laboratory only defined the RI of PINP in premenopausal females, we compared it with the results previously reported using the Roche electrochemiluminescence system.[16]

Two levels of quality control were implemented for all test items before and after each batch test, and the samples would be tested only after the results of quality control were qualified. In addition, the laboratory of Peking Union Medical College Hospital is a double-certified Laboratory of CAP and ISO15189. All of the above testing items also participated in the external quality assessment of the Clinical Laboratory Center of National Health and Health Commission in China, and the results were satisfactory.

### 2.3 | Statistical analysis

SPSS 22.0 (IBM Inc.,) and GraphPad Prism 7.0 (GraphPad Software) were used for data analysis and presentation. Multiple regression analysis (MRA) was used to determine the effects of the major indices, including altitude, sex, age, BMI, and SBP, on bone metabolism-associated biomarkers. Group A (Ali region), male, aged 19 to 29 years, was set as the reference group for the analysis of altitude, sex, and age, respectively. In addition, BMI and SBP were analyzed as continuous variables. Standard partial correlation coefficients (*rp*) were calculated and evaluated according to a method described in a previous report.[17] The Mann-Whitney *U* test or Kruskal-Wallis test was used for intergroup comparisons. Pearson's correlation analysis was used for normally distributed data, whereas Spearman's correlation analysis was used for non-normally distributed data to calculate the correlation coefficients (*r*). Notably, *r* values between 0.36 and 0.67 were considered to represent moderate correlations, whereas those between 0.68 and 1.0 were considered as strong correlations.[18] The distribution of bone metabolism-associated biomarkers according to age was fitted via the Loess method by SPSS 22.0. Two-sided $p$ value <0.05 was considered to be statistically significant.

## 3 | RESULTS

### 3.1 | Participant characteristics

In total, 1246 participants (mean age: 39 ± 11 years, male: $n = 543$) were enrolled in this study. Postmenopausal individuals (according to questionnaire) accounted for 23.7% of the female population, with a higher age distribution (mean age: 55 ± 5 years vs. 35 ± 9 years). Of 1246 individuals, 18.6% of participants had the habit of smoking, 25% of participants were on a vegetarian diet, and 19.7% of participants were on night shift. The characteristics of the individuals grouped according to the altitude at the place of residence and the sex are shown in Table 1. There were significant differences in the variables, except for the urea levels ($p = 0.470$), among individuals from the different altitude groups. The age distribution between males and females did not differ significantly ($p = 0.296$); however, BMI, blood pressure, and the levels of Alb, ALT, TG, TC, glucose, and urea were significantly higher in males than in females (all $p < 0.05$).

### 3.2 | Correlation and regression analysis

MRA was performed to evaluate the influence of common factors on bone metabolism-associated biomarkers, and the results of the standardized *rp* are summarized in Table 2. Sex, altitude, age, and BMI but not blood pressure were the major factors that

**TABLE 1** Participant characteristics based on altitude and sex

| | Group A | | Group B | | Group C | |
|---|---|---|---|---|---|---|
| **Variables** | **Male ($n = 213$)** | **Female ($n = 223$)** | **Male ($n = 207$)** | **Female ($n = 249$)** | **Male ($n = 123$)** | **Female ($n = 231$)** |
| Age (y) | 36 ± 10 | 36 ± 10 | 40 ± 12 | 42 ± 12 | 42 ± 11 | 41 ± 12 |
| BMI (kg/m$^2$) | 23.6 ± 3.5 | 22.6 ± 4.0 | 24.8 ± 3.8 | 24.0 ± 3.9 | 26.0 ± 3.6 | 24.4 ± 4.1 |
| SBP (mmHg) | 114 ± 17 | 107 ± 18 | 122 ± 17 | 117 ± 16 | 124 ± 18 | 121 ± 21 |
| DBP (mmHg) | 74 ± 15 | 72 ± 13 | 85 ± 14 | 80 ± 12 | 83 ± 14 | 77 ± 14 |
| Alb (g/L) | 49.2 ± 3.1 | 47.1 ± 3.2 | 50.0 ± 3.3 | 47.8 ± 3.1 | 46.9 ± 2.7 | 46.3 ± 2.6 |
| ALT (U/L) | 36 (25,57) | 20 (14,29) | 31 (21,50) | 18 (13,27) | 32 (22,49) | 20 (15,29) |
| TG (mmol/) | 1.01 (0.71,1.65) | 0.70 (0.55,1.02) | 1.12 (0.85,1.50) | 0.86 (0.60,1.20) | 1.23 (0.92,1.72) | 0.86 (0.63,1.19) |
| TC (mmol/L) | 4.74 ± 1.02 | 4.34 ± 0.76 | 4.95 ± 0.97 | 4.72 ± 0.91 | 4.79 ± 0.85 | 4.63 ± 0.93 |
| Glucose (mmol/L) | 4.99 ± 0.86 | 4.71 ± 0.53 | 4.56 ± 1.10 | 4.39 ± 0.93 | 4.88 ± 1.11 | 4.66 ± 0.85 |
| Urea (mg/dL) | 4.68 ± 1.25 | 4.34 ± 1.25 | 4.69 ± 1.33 | 4.31 ± 1.04 | 4.66 ± 1.29 | 4.25 ± 1.14 |

*Note:* Age, BMI, SBP, DBP, and the levels of Alb, TC, glucose, and urea are presented as mean ± SD; ALT and TG are presented as medians (25th and 75th percentiles).

Abbreviations: Alb, Albumin; ALT, Alanine transaminase; BMI, Body mass index; DBP, Diastolic blood pressure; SBP, Systolic blood pressure; TC, Total cholesterol; TG, Triglyceride.

**TABLE 2** Standard partial correlation coefficients from multiple regression analysis

| Variables | R-value | Group B | Group C | Sex | 30–39 years | 40–49 years | 50–64 years | BMI | SBP |
|---|---|---|---|---|---|---|---|---|---|
| Serum calcium | 0.369 | **0.173*** | **−0.128*** | **−0.160*** | **−0.116*** | **−0.172*** | −0.064 | **−0.083*** | 0.009 |
| Serum phosphorus | 0.405 | **0.185*** | **−0.093*** | **0.206*** | **−0.213*** | **−0.171*** | −0.014 | −0.056 | **−0.080*** |
| ALP | 0.275 | 0.001 | −0.060 | **−0.176*** | −0.032 | −0.042 | **0.081*** | **0.097*** | 0.062 |
| 25OHD | 0.554 | −0.058 | **−0.141*** | **−0.525*** | 0.016 | −0.023 | **0.083*** | **−0.060*** | 0.042 |
| PINP | 0.381 | **−0.088*** | 0.026 | **−0.158*** | **−0.321*** | **−0.346*** | **−0.201*** | −0.067 | −0.015 |
| β-CTX | 0.413 | **−0.305*** | **−0.151*** | **−0.196*** | **−0.264*** | **−0.221*** | −0.062 | **−0.091*** | 0.015 |
| PTH | 0.463 | **0.172*** | **0.449*** | **0.167*** | 0.027 | 0.016 | −0.040 | **0.130*** | 0.020 |

Abbreviations: 25OHD, 25 Hydroxyvitamin D; ALP, Serum alkaline phosphatase; BMI, Body mass index; PINP, Type I collagen amino terminal elongation peptide; PTH, Parathyroid hormone; SBP, Systolic blood pressure; β-CTX, β-collagen degradation products.

*$p < 0.05$.

affected bone metabolism-associated biomarkers. In addition, the levels of urine calcium and urine phosphorus were measured and corrected according to the urine Cr levels in this study. The correlation analysis indicated a weak correlation between urine phosphorus/Cr and serum phosphorus levels ($r = 0.151$), urine calcium/Cr and urine phosphorus/Cr levels ($r = 0.187$), and serum calcium and serum phosphorus levels ($r = 0.323$), a moderate correlation between ALP and PINP levels ($r = 0.440$), and ALP and CTX levels ($r = 0.366$), and a strong correlation between PINP and CTX levels ($r = 0.745$).

Since the MRA results indicated that BMI was an important factor that affected bone metabolism-associated biomarkers, we evaluated the distribution of these biomarkers in different BMI groups (Figure S1). The levels of serum calcium, serum phosphorus, PINP, and CTX decreased with an increase in BMI; however, the reduction in the serum phosphorus levels was not significant ($p = 0.134$ in males; $p = 0.179$ in females). In contrast, the levels of ALP in females and PTH in both males and females increased with the increase in BMI; however, no significant linear trends were observed in the distribution of ALP levels in males and 25OHD levels in both males and females.

## 3.3 | Distribution of bone metabolism-associated biomarkers based on altitude and sex

As shown in Table 1, the age distribution differed among individuals from different regions but not between males and females; therefore, individuals from the three altitude groups were randomly resampled based on the age groups (age, 19–29: 30–39: 40–49: 50–64 years =1:1:1:1) to modify the effect of age in the different regions. As shown in Table 3, there were significant differences in the distribution of serum calcium, serum phosphorus, 25OHD, CTX, and PTH levels among the different altitude groups, but these differences did not apply to the ALP and PINP levels. The ALP and 25OHD levels decreased and the PTH levels increased with decreasing altitude. The serum calcium and phosphorus levels were the highest, and the PINP and CTX levels were the lowest in altitude Group B. Moreover, the levels of all biomarkers differed significantly between males and females ($p < 0.05$ for all). The serum phosphorus and PTH levels were higher in females, whereas the levels of the five other biomarkers were significantly higher in males. Compared with the distribution of serum calcium and phosphorus levels, the distribution of urine calcium and phosphorus levels exhibited greater interindividual variation. The ratios of calcium and phosphorus levels to Cr levels in the total population were 197 (112, 281) and 1384 (982, 1823), respectively. Females showed significantly higher levels of these two indicators than males (median urinary calcium/Cr: 213 vs. 181, median urinary phosphorus/Cr: 1525 vs. 1238; $p < 0.05$ for all).

## 3.4 | Distribution of bone metabolism-associated biomarkers according to age

Fitting was performed using the Loess method. The distribution of bone metabolism-associated biomarkers with age in males and females (individually) is shown in Figure 1. The serum levels of phosphorus, ALP, PINP, and CTX were closely associated with the aging process; in general, the levels declined persistently with age in males, whereas in females, the levels initially decreased with age, reached a trough at ages close to 40 years, and then increased with age. Overall, the levels of ALP, PINP, and CTX were higher in males aged less than 50 years, whereas the trend reversed after the age of 50. The changes in the levels of the bone formation marker PINP and the bone resorption marker CTX with age were consistent. At the same time, the PTH levels in females and the serum calcium and 25OHD levels in both males and females showed a stable distribution with age. Furthermore, the levels of serum calcium [(2.43 ± 0.13) vs. (2.39 ± 0.12) mmol/L), serum phosphorus ((1.25 ± 0.17) vs. (1.17 ± 0.16) mmol/L), ALP ((121.7 ± 35.6) vs. (91.8 ± 25.5) U/L), PINP (72.8 (60.6, 94.4) vs. 56.5 (43.0, 74.7) ng/ml), and CTX (0.51 (0.39, 0.67) vs. 0.37 (0.28, 0.51)] ng/ml were significantly higher in postmenopausal females than in premenopausal females.

TABLE 3 Distribution of bone metabolism-associated biomarkers based on altitude and sex

| Variables | Group A | | Group B | | Group C | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Male ($n$ = 95) | Female ($n$ = 105) | Male ($n$ = 179) | Female ($n$ = 201) | Male ($n$ = 94) | Female ($n$ = 166) | Male ($n$ = 268) | Female ($n$ = 472) |
| Calcium (mmol/L) | 2.45 ± 0.12* | 2.38 ± 0.13 | 2.50 ± 0.13* | 2.45 ± 0.14 | 2.36 ± 0.09 | 2.39 ± 0.11 | 2.45 ± 0.13# | 2.41 ± 0.13# |
| Phosphorus (mmol/L) | 1.11 ± 0.13* | 1.19 ± 0.19 | 1.19 ± 0.17* | 1.22 ± 0.17 | 1.02 ± 0.15* | 1.17 ± 0.16 | 1.13 ± 0.17# | 1.20 ± 0.17# |
| ALP (U/L) | 117.8 ± 33.2* | 92.1 ± 26.9 | 112.9 ± 29.5* | 102.5 ± 32.2 | 107.2 ± 28.6 | 99.5 ± 28.6 | 112.7 ± 30.4# | 99.2 ± 30.0# |
| 25OHD (ng/ml) | 25.6 ± 9.6* | 16.7 ± 6.4 | 24.7 ± 7.8* | 15.0 ± 5.9 | 21.5 ± 6.3* | 14.2 ± 5.4 | 24.0 ± 8.0# | 15.0 ± 5.9# |
| PINP (ng/ml) | 70.9 (34.2, 166.5)* | 60.9 (21.7, 174.0) | 68.8 (28.5, 158.8)* | 62.2 (22.0, 158.0) | 74.0 (29.70, 188.6) | 66.5 (29.9, 161.8) | 69.3 (31.0, 166.5)# | 62.1 (22.8, 159.1) |
| β-CTX (ng/ml) | 0.61 (0.24, 1.23)* | 0.50 (0.17, 1.20) | 0.46 (0.22, 1.02)* | 0.35 (0.15, 0.95) | 0.50 (0.18, 1.12) | 0.44 (0.15, 1.00) | 0.50 (0.22, 1.16)# | 0.40 (0.16, 1.03)# |
| PTH (pg/ml) | 33.9 (18.2, 73.8)* | 38.0 (19.5, 60.8) | 38.1 (18.3, 75.5)* | 44.1 (21.9, 81.3) | 49.4 (24.7, 93.7)* | 54.1 (28.2, 105.6) | 40.4 (20.5, 83.9)# | 45.8 (22.2, 90.7)# |

Abbreviations: ALP, Serum alkaline phosphatase; 25OHD, 25 Hydroxyvitamin D; PINP, type I collagen amino terminal elongation peptide; β-CTX, β-collagen degradation products; PTH, parathyroid hormone. Calcium, phosphorus, ALP, and 25OHD levels are presented as mean ± SD; PINP, β-CTX, and PTH levels are presented as medians (2.5th and 97.5th percentiles).

*Meant significant difference between sex in different altitude groups

#Meant significant difference among altitude groups in different sex.

### 3.5 | Evaluation of the bone metabolism status in Tibetan adults

Based on the RIs shown in Table S1, the expression of abnormal bone metabolism-associated biomarkers in Tibetan adults was evaluated in this study (shown in Figure 2). The proportion of abnormalities in the levels of serum calcium, serum phosphorus, PINP, and PTH was higher in females than in males; in contrast, the proportion of abnormalities in the levels of ALP and CTX was lower. The percentages of 25OHD deficiency, insufficiency, and sufficiency were 33.2%, 45.8%, and 21.0% in males and 83.1%, 14.6%, and 2.2% in females, respectively.

Furthermore, we also observed that individuals with a smoking habit had higher levels of ALP, PINP, and CTX than those without smoking habits (mean ALP levels: 114.4 vs. 102.4 U/L; median PINP levels: 74.4 vs. 62.8 ng/ml; median CTX levels: 0.51 vs. 0.44 ng/ml). Additionally, vegetarian individuals (mean ALP levels: 106.0 vs. 104.4 U/L; PINP levels: 66.9 vs. 63.0 ng/ml; CTX levels: 0.47 vs. 0.44 ng/ml) and those working in night shifts (ALP levels: 106.2 vs. 104.7 U/L; PNP levels: 67.2 vs. 61.8 ng/ml; CTX levels: 0.45 vs. 0.43 ng/ml) had slightly higher levels of ALP, PINP, and CTX than nonvegetarian individuals or individuals working in day shifts, respectively.

## 4 | DISCUSSION

A previous study showed that prolonged residency at extreme altitudes (3450–6700 m) for approximately 4 months to 1 year would lead to the deterioration of skeletal health.[19,20] Basic studies also reported that sustained and intermittent hypoxia could induce the differentiation and mineralization of osteoblast progenitors, leading to bone loss.[10,21,22] However, compared with previous multicenter studies aimed at individuals living in the plain areas of China,[23] we found that Tibetan individuals had significantly high levels of PINP and CTX (PINP: 65.3 vs. 49.36 ng/ml, CTX: 0.46 vs. 0.37 ng/ml) with similar levels of 25OHD,[23,24] which implied that the Tibetan individuals recruited in this study had more active bone metabolism. With the consumption of a calcium-enriched diet containing meat and dairy products and sufficient light exposure, especially with routine outdoor pilgrimage, previous studies aimed at Tibetan individuals also found significantly higher BMD and a lower prevalence of osteoporosis.[12–14] Thus, we postponed the idea that extreme altitude could accelerate the process of osteoclasts, while certain genetic or metabolic changes, such as variants of hypoxia-inducible factors and bone morphogenetic proteins, might have occurred in native Tibetans to attenuate the damage.[25,26] Further studies are still necessary to explore the underlying mechanism.

The highest levels of serum calcium and serum phosphorus and the lowest levels of PINP and CTX were found in individuals from altitude Group B, which implied that the bone metabolism status in Group B (Lhasa and Shigatse) was better than that in Group A (Ali) and Group C (Nyingchi). This finding could be related to the higher level of urbanization and the higher quality of life in Lhasa and

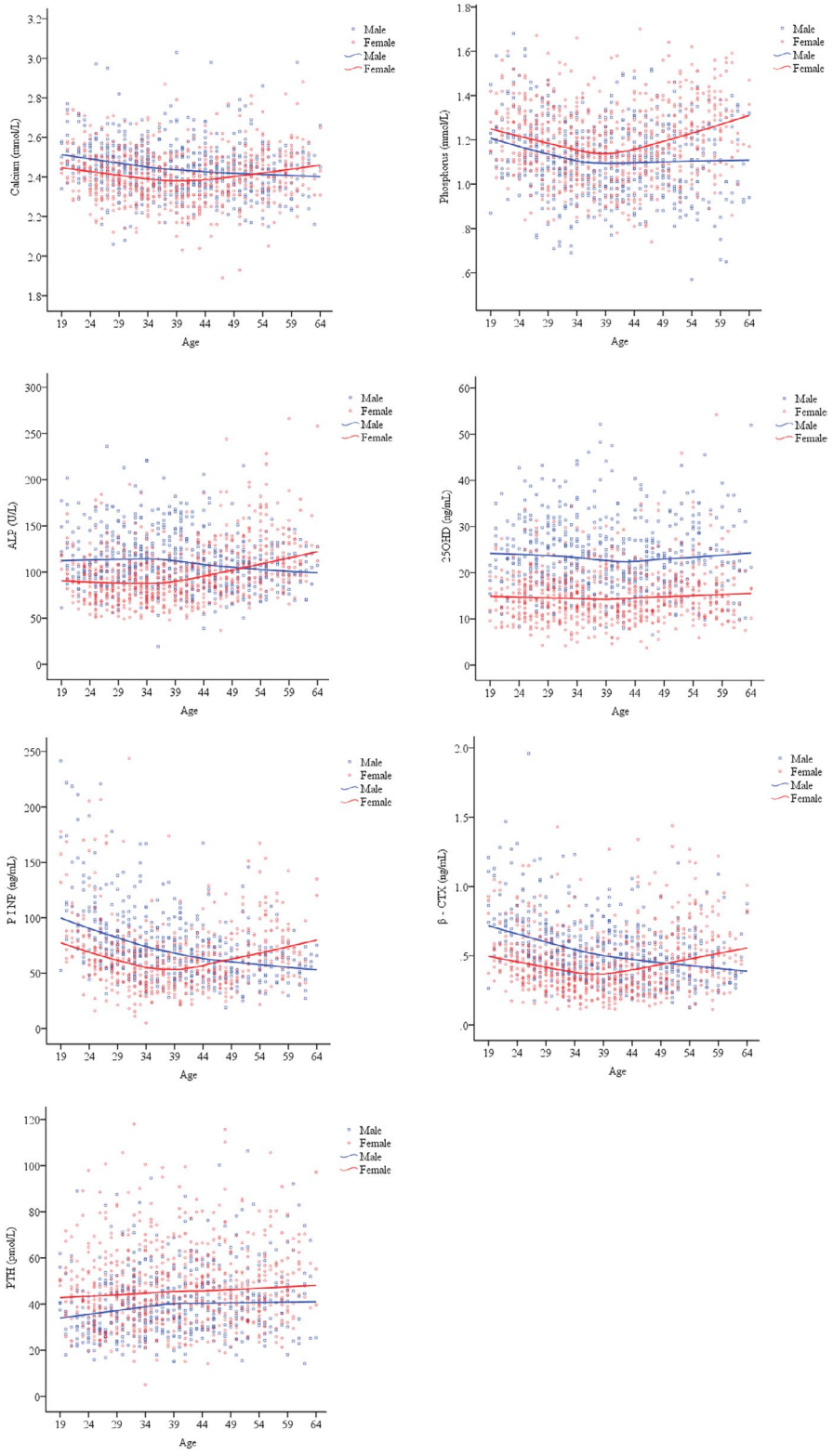


**FIGURE 1** Distribution of bone metabolism-associated biomarkers with age. ALP, serum alkaline phosphatase; 25OHD, 25 Hydroxyvitamin D; PINP, type I collagen amino terminal elongation peptide; β-CTX, β-collagen degradation products; PTH, parathyroid hormone

Shigatse. Moreover, the levels of 25OHD tended to increase with increasing altitude, which was consistent with a previous report and could be related to the increase in ultraviolet intensity alongside the increase in altitude.[27]

Affected by sex hormone levels, including insulin-like growth factor-I levels, the state of bone turnover was more active in young males and decreased with age, which is consistent with findings from previous studies.[16,28,29] However, for females, the active state of bone turnover increased significantly before approximately 40 years old, when most women enter the perimenopausal stage with a considerable reduction in estrogen levels. In contrast to findings from previous studies,[23] the increase in ALP, PINP, and CTX in females occurred earlier, which could be associated with a shorter life expectancy leading to earlier menopause in the Tibetan population.

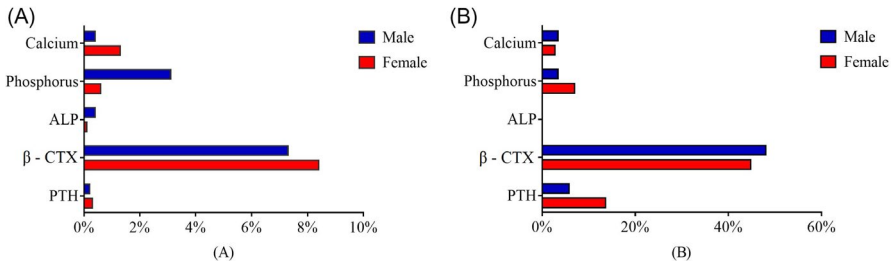


**FIGURE 2** Prevalence of abnormal bone metabolism-associated biomarkers in Tibetan adults. ALP, serum alkaline phosphatase; PINP, type I collagen amino terminal elongation peptide; β-CTX, β-collagen degradation products; PTH, parathyroid hormone. (A) indicates that the levels of these biomarkers were lower than the lower limit of RIs; (B) indicates that the levels were higher than the upper limit of the RIs

Furthermore, based on the RIs from the manufacturer and a previous study,[16] more than 50% of individuals among both males and females had abnormal levels of PINP and CTX, which suggested that RIs should be specially established for the Tibetan population.

In this study, the levels of PINP and CTX decreased with increasing BMI, which indicated a positive correlation between BMI and bone metabolism status. However, the association and specific interaction between BMI and bone metabolism remain contradictory and unclear; thus, further investigation is still required.[30–33] Furthermore, we observed that not only individuals with a smoking habit[34] but also those with vegetarian habits or working at night shifts had higher levels of ALP, PINP, and CTX than those who did not. The results implied that certain habits, especially smoking, could be detrimental to bone metabolism and could serve as risk factors for osteoporosis.

To our knowledge, this study is the first multicenter study to comprehensively evaluate the distribution and major factors affecting the levels of seven bone metabolism-associated biomarkers in the Tibetan region. Detailed information on nationality, health conditions, and daily habits was derived using standard questionnaires. However, the study did not collect and measure specific information, such as BMD and long-term drinking habits, which should be investigated in future studies. Since the Tibetan people are nomadic and have their own religious beliefs, it is difficult to recruit volunteers and measure their BMD, especially in the Ali region. Moreover, the age distribution in the different altitude groups was significantly different ($p < 0.001$), but it is consistent with the overall distribution in the Tibetan region, as indicated in the sixth census of China.[35] With a typical early adulthood age structure, it was even more difficult to recruit older individuals from Ali. Thus, we randomly sampled participants based on age groups to offset the effect. Last, since the results obtained using different measurement assays have been shown to differ significantly,[36] the distribution of bone metabolism-associated biomarkers obtained in this study was more applicable for Roche E601 electrochemiluminescence analyzers.

In conclusion, a more active bone turnover status was found in Tibetan adults than in the plain area of China, with higher levels of ALP, PINP, and CTX. Altitude, sex, age, and BMI affected the status of bone metabolism, as did smoking, vegetarianism, and work in night shift. Thus, subgroup-specific RIs must be established for the correct evaluation of bone metabolism.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## AUTHOR CONTRIBUTIONS

Yutong Zou mainly wrote this article; Zhijuan Liu, Honglei Li, Li'an Hou, Jinrong Pang, Xiaoxing Liu, Zejipuchi, Liping Tian, and Hongyan Yang mainly conducted this multicenter cross-sectional research; Songlin Yu, Danchen Wang, Xiuzhi Guo, and Xinqi Cheng finish and check all clinical laboratory measurements; Qi Zhang and Chaochao Ma help to analyze the data; Ling Qiu is the planner and tutor of this research.

## ETHICAL APPROVAL

This study was approved by the Ethics Committee of People's Hospital of Tibet Autonomous Region (ME-TBHP-2017–021), and all participants provided written informed consent for participation in the study. This study was also approved by the Ethics Committee of Peking Union Medical College and Chinese Academy of Medical Sciences at Peking Union Medical College Hospital (S-K530).

## DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article [and/or] its supplementary material files. Further enquiries can be directed to the corresponding author.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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