Osteoporosis and Sarcopenia 9 (2023) 131–136

Contents lists available at ScienceDirect

Osteoporosis and Sarcopenia

journal homepage: www.elsevier.com/locate/afos

Original article

Bone mineral metabolism and different indices of skeletal health of Ladakhi women living at high altitude

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ARTICLE INFO

Keywords:
High altitude
Ladakhi
Osteoporosis
Skeletal health
Bone mineral density

ABSTRACT

Objectives: High altitude possesses a great challenge for human survival owing to low oxygen tension and has been reported to cause bone deterioration among sojourns of high altitude. The bone health of Ladakhi women is investigated for the first time in this study.

Methods: Dual energy X-ray absorptiometry of Ladakhi women and sea level women was done at the radius and calcaneus using EXA-3000 (Osteosys, Korea), followed by colorimetric and Enzyme Linked Immunosorbent Assay analysis of parameters regulating bone health.

Results: There was no statistically significant difference between bone mineral density of Ladakhi women and sea level women at radius ($P = 0.287$) or calcaneus ($P = 0.839$). Almost similar cases of osteopenia were reported at both sites measured in the study among both groups. Two post-menopausal Ladakhi women however, had osteoporosis at the radius while 4 had osteoporosis at calcaneus. Significant increase in calcium levels with a decrease in intact parathyroid hormone and an increase in calcitonin levels were observed in Ladakhi women as compared to sea level women. Though there was no significant difference in 25-hydroxy vitamin D levels of both groups, a higher percentage of 25-hydroxy vitamin D deficiency (77% *vs* 23%) was observed in Ladakhi women as compared to sea level women. Estradiol levels were similar in both groups.

Conclusions: The present study suggest that there is no significant relationship between high altitude living and bone mineral density among Ladakhi women.

1. Introduction

Nearly 81.6 million people live permanently in high-altitude (HA) regions while others are visiting them for professional and recreational purposes [1]. Survival in extreme cold, high wind velocity, dehydration, solar radiation, malnutrition and especially hypobaric hypoxia of HA environment requires adjustments across various organ systems through acclimatization [2]. As reviewed extensively by Babu and Ghosh in "Looking at mountains: Role of Sustained Hypoxia in Regulating Bone Mineral Homeostasis in Relation to Wnt Pathway and Estrogen". Hypoxia has also been associated with significant decline in bone mineral density (BMD) accompanied with changes in hormonal and biochemical indicators of bone remodeling in low landers exposed to high altitude (HA). Reduced BMD along with decline in both bone formation markers namely alkaline phosphatases (ALP), bone specific alkaline phosphatases (BAP), calcitonin (CT) and bone resorption markers namely C-Telopeptide of Type I collagen, N-Terminal Telopeptide reflecting a lower bone turnover, contributing to significant amount of osteopenia and osteoporosis was reported in soldiers after stay at high and extreme altitude for 40 and 16 weeks, respectively. The bone strength parameters such as maximum load, stiffness, young's modulus were significantly reduced at simulated altitudes in rat models as well. Low oxygen tension has been reported to inhibit the formation and activity of osteoblasts thereby limiting bone formation and stimulating bone resorption by accelerating formation and activity of osteoclasts in many reports but contradictory results have also been reported [3].

Early identification followed by appropriate intervention among subjects at high risk of osteoporosis and related morbidity can reduce considerable amount of economic and social burden of the condition. Identification of individuals at risk of osteoporosis requires accurate measurement of BMD and comparison to a young normal reference population [4]. Natives of HA may represent long term effects of

Peer review under responsibility of The Korean Society of Osteoporosis.

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https://doi.org/10.1016/j.afos.2023.11.001

Received 25 July 2023; Received in revised form 26 October 2023; Accepted 16 November 2023

Available online 29 November 2023

prolonged HA residency on bone mineral metabolism. To date, knowledge about effect of HA environment on bone health can be attributed to few studies reported mainly on male volunteers [5–8]. We have, therefore, performed cross sectional analysis of bone health indicators and regulators in Ladakhi women (LDK), native to 3500 m and compared it with age-matched sea level women (SLN), residing at nearly 200 m. To our knowledge, this is the first study trying to establish bone health status of pre-menopausal women of HA region. The aim of this study is to compare BMD along with biochemical and endocrinal regulators of bone health in LDK to establish bone health status of Ladakhi women.

2. Methods

2.1. Ethical approval

The proposed study protocol-EC/DIPAS/B-6/2/2015 was approved by the Institute's Ethics Committee as per Helsinki declaration and its later amendments. The bone health indicator studies were carried out in LDK and SLN groups consisting of healthy pre-menopausal women of 20–50 years of age. The experimental procedure was explained to all volunteers prior to experimentation and written consent was obtained. General information such as name, date of birth, ethnicity, address, education, occupation, lifestyle, dietary habits, exercise protocol, medication history, history of familial osteoporosis, and any occurrence of stress fracture was recorded.

2.2. Study participants

LDK group consisted of volunteers native to Leh, Ladakh (3500 m) and various study parameters were collected at Defence Institute for High Altitude Research (DIHAR), Leh, India. SLN group consisted of aged matched volunteers having similar socio-economic background, residing in Delhi and parameters were recorded at Defence Institute of Physiology and Allied Sciences, Delhi, India. Both groups comprised of research scholars, scientists, and other staffs from the institutes, and their lifestyle was mostly sedentary. A total of 47 SLN and 48 LDK volunteered for the study but only healthy pre-menopausal volunteers with no obvious signs and symptoms of metabolic bone disease and/or any other illness such as diabetes mellitus, parathyroidism, thyroidism, lactose intolerance, impaired renal, hepatic, or reproductive function, and were not taking any supplement affecting bone mineral metabolism were included in the study. Volunteers who had not been to HA for minimum of 1 year were only included in the SLN group. All volunteers followed normal daily routine and diet while being part of the study. Physical data was obtained with minimum clothing and without shoes. Height and weight were recorded using scales and body mass index (BMI) was calculated accordingly.

2.3. BMD measurement

BMD was assessed by measurements taken at the ultra-distal radius of non-dominant arm and left calcaneus using peripheral dual energy X-ray absorptiometry (DXA) using an "EXA-3000 (Osteosys, Seoul, Korea)" instrument, according to the protocol mentioned by the manufacturer. Quality control procedures were also carried out in accordance with the manufacturer's guidance. T-score was obtained from software "Osteosys- EXA" provided with the machine. It was based on reference values of BMD forearm and BMD calcaneus of Asian database. Assessment of T-score was carried out using standards defined by World Health Organization (WHO) for determining bone health status. The criteria are defined as ''the T-score'' between −1 and −2.5 termed as ''osteopenia'' and less than −2.5 termed as ''osteoporosis".

2.4. Biochemical and hormonal parameter analysis

Fasting venous blood samples were drawn from each volunteer into Serum Separator Tubes (367956, SST_ II Advance; BD) in the morning. Whole blood in SST was centrifuged at 3000 rpm for 15 min at room temperature to obtain the serum which was then aliquoted and stored at −80°C for further analysis. All parameters were measured in duplicates using commercially available kits in accordance to manufacturer's guidelines in a multi-plate reader (Tecan Infinite 200 Pro, Switzerland).

1. Estimation of serum calcium

The serum calcium levels were measured using Calcium colorimetry kit (D01377, Dilab, Austria) according to manufacturer's instructions. The kit measured calcium up to 20 mg/dL with lower limit of detection up to 0.04 mg/dL. Briefly, 10 μL of standard (10 mg/dL) and each serum samples were mixed with 1000 μL of reagent and incubated at room temperature for 5 minutes. Absorbance was read at OD650 nm using micro-plate reader against reagent blank containing 10 μL distilled water. The results were analyzed against the concentration of standard.

2. Estimation of serum phosphorus

The serum phosphorus levels were measured using phosphorus colorimetry kit (D01377, Dilab, Austria) according to manufacturer's instructions. The kit measured phosphorus up to 15 mg/dL with lower limit of detection up to 0.07 mg/dL. Briefly, 10 μL of standard (5 mg/dL) and each serum samples were mixed with 1000 μL of reagent and incubated at room temperature for 5 minutes. Absorbance was read at OD650 nm using micro-plate reader against reagent blank containing 10 μL distilled water. The results were analyzed against the concentration of standard.

3. Estimation of serum BAP

The serum BAP levels were measured using BAP EIA kit (MicroVue BAP EIA, Quidel, San Diego, CA, USA) as per manufacturer's instructions. The kit had an assay range of 2–140 U/L and sensitivity of 0.7 U/L. Briefly, 20 μL of standards, controls and each serum sample were mixed with 125 μL of assay buffer and incubated at room temperature for 3 hours. The plate was washed and 150 μL of substrate was added to each well and incubated for 30 minutes at room temperature. Finally, 100 μL of stop solution was added to each well and absorbance was read at OD405 nm using microplate reader. Results were analyzed using a quadratic curve fit.

4. Estimation of serum 25-OH vitamin D

The serum 25-OH vitamin D levels were measured using total 25-OH vitamin D EIA kit (KT715, EDI, USA) as per manufacturer's instructions. The kit had an assay range of 0–115 ng/ml and sensitivity of 5 ng/ml. 20 μL of standards, controls and each serum sample were mixed with 100 μL of vitamin D assay buffer and incubated at room temperature for 1 hour. 25 μL Biotinylated vitamin D analogue was added to each well and incubated at room temperature for 30 minutes. The plate was washed and 100 μL of Streptavidin-HRP was added to each well and incubated at room temperature for 20 minutes. Plate was again washed and 100 μL HRP substrate was added to each well and incubated for 20 minutes at room temperature. Finally, 100 μL of stop solution was added to each well and absorbance was read at OD450 nm using microplate reader. Results were analyzed using four-parameter calibration curve fitting.

5. Estimation of serum i-PTH

The serum i-PTH levels were measured using i-PTH ELISA kit (EIA-3645, DRG International, Marburg, Germany) as per manufacturer's

instructions. The kit had assay range of 1.57–210 pg/mL and sensitivity of 1.57 pg/mL. 25 μL of standards, controls and each serum sample were mixed with 50 μL each of biotinylated antibody and enzyme labeled antibody. The plate was incubated at room temperature for 3 hours. The plate was washed and 150 μL of TMB substrate was added to each well and incubated for 30 minutes at room temperature. Finally, 100 μL of stop solution was added to each well and absorbance was read at OD450 nm using microplate reader. Results were analyzed using four-parameter calibration curve fitting.

6 Estimation of serum calcitonin

The serum calcitonin levels were measured using calcitonin ELISA kit (EIA-3648, DRG International, Germany) as per manufacturer's instructions. The kit had assay range of 1–1000 pg/mL and sensitivity of 1 pg/mL. 100 μL of standards, controls and each serum sample were mixed with 50 μL each of biotinylated antibody and enzyme labeled antibody. The plate was incubated at room temperature for 4 hours. The plate was washed and 150 μL of TMB substrate was added to each well and incubated for 30 minutes at room temperature. Finally, 100 μL of stop solution was added to each well and absorbance was read at OD450 nm using microplate reader. Results were analyzed using four-parameter calibration curve fitting.

7 Estimation of serum estradiol

The serum estradiol levels were measured using estradiol ELISA kit (EIA-2693, DRG International, Germany) as per manufacturer's instructions. The kit had an assay range of 10.6–2000 pg/mL and sensitivity of 10.6 pg/mL. 25 μL of standards, controls and each serum sample were mixed with 100 μL of enzyme conjugate. The plate was incubated at room temperature for 1.5 hours. The plate was washed and 100 μL of substrate was added to each well and incubated for 30 minutes at room temperature. Finally, 50 μL of stop solution was added to each well and absorbance was read at OD450 nm within 10 minutes using microplate reader. Results were analyzed using four-parameter calibration curve fitting.

2.5. Statistical analysis

All data collected (physiological, bone densitometry, biochemical and hormonal parameters) was entered into Microsoft Excel file. Mean and Standard error of mean (SEM) were calculated for all sets of data and student's *t*-test was used to compare the means of LDK and SLN group. Probability values equal to or less than 0.05 and 0.01 were considered statistically significant and highly significant difference between the groups, respectively.

3. Results

Altogether 95 (47 SLN and 48 LDK) consenting women volunteered for the study among which 63 (34 SLN and 29 LDK) were found eligible for the study. Nineteen (4 SLN and 15 LDK) post-menopausal women were excluded from the study. However, BMD of 15 postmenopausal LDK women was analyzed separately. Twelve (8 SLN and 4 LDK volunteers) were removed for having metabolic conditions affecting bone mineral metabolism and 1 SLN subject visited HA during the study. No volunteers of both groups showed any sign of osteomalacia. Table 1 shows the physiological features of the 2 groups. Mean age of SLN group was similar to LDK group. LDK had significantly reduced height ($P < 0.001$) and weight ($P = 0.02$) compared with their SLN counterparts. Mean height of LDK and SLN group were 152.40 ± 4.57 and 160.45 ± 6.24, respectively. Similarly, mean weight of LDK and SLN were 56.61 ± 8.52 and 62.24 ± 9.34, respectively. However, there was no change in BMI between the groups. LDK and SLN groups had BMI of 24.18 ± 2.56 and 24.98 ± 4.03, respectively.

Table 1
Physiological features of subjects.

Parameters	Sea Level Women Mean ± SD	Ladakhi Women Mean ± SD	P-value	Effect Size (Hedges' G)
Age, yr	34.29 ± 7.70	35.72 ± 5.83	0.423	0.207
Height, cm	160.45 ± 6.24	152.40 ± 4.57	0.000**	1.530
Weight, kg	62.24 ± 9.34	56.61 ± 8.52	0.02*	0.6628
Body Mass Index, kg/m^2	24.98 ± 4.03	24.18 ± 2.56	0.380	0.233

Values represented as mean ± SD unless stated otherwise. Significance of changes in the mean values of Ladakhi women and sea level women was determined by Student's *t*-test (**P < 0.01; *P < 0.05); SD, standard deviation, years; centimeters; kg-kilogram; kg/m^2-kilograms per square meter.

Table 2 shows the mean BMD along with cases of osteopenia and osteoporosis at ultra-distal radius of non-dominant arm and left calcaneus in LDK and SLN groups. The mean BMD at radius in the LDK was 0.49 ± 0.06 g/cm^2 as compared to 0.47 ± 0.05 g/cm^2 of SLN. Similarly, the mean BMD at calcaneus for both LDK and SLN subjects were 0.49 g/cm^2 each. No significant change in BMD was found at either site measured in this study. Five LDK were reported to have osteopenia at radius while 6 had osteopenia at calcaneus as compared to 4 SLN with osteopenia at radius and 5 with osteopenia at calcaneus. In summary, equal number of volunteers suffered from osteopenia in SLN and LDK at both sites measured in the study. The mean BMD of 15 post-menopausal Ladakhi women at radius and calcaneus were 0.41 ± 0.10 g/cm^2 and 0.44 ± 0.10 g/cm^2, respectively. Four post-menopausal LDK volunteers suffered from osteoporosis in radius while 2 had osteoporosis at calcaneus. One subject was also reported to have osteopenia at the calcaneus.

Table 4 depicts serum concentrations of biochemical and endocrinal parameters related to bone health. Calcium and phosphorus (P) together form hydroxyapatite, the most abundant and inorganic component of bone. There was a significant increase ($P = 0.045$) in mean serum Ca levels of LDK (9.87 ± 0.75 mg/dL) as compared to SLN (9.37 ± 0.98 mg/dL). However, mean serum P levels were similar for LDK (3.34 ± 0.72 mg/dL) and SLN (3.55 ± 0.75 mg/dL groups.

BAP and 25 vitamin D play important role in bone formation. There were no significant differences in levels of BAP and 25 vitamin D in LDK and SLN groups. The mean serum BAP levels for LDK and SLN groups were 23.37 ± 4.42 U/L and 20.40 ± 6.73 U/L, respectively. The mean serum 25 vitamin D levels for LDK and SLN groups were 14.64 ± 6.79 ng/mL and 20.86 ± 8.38 ng/mL, respectively. Table 3 depicts the 25 vitamin D deficiency statuses of the subjects. Only 23% of the SLN group

Table 2
Bone Mineral Density and bone heath status of subjects.

Parameters	Pre-Menopausal		Post-Menopausal
	Sea Level Women	Ladakhi Women	Ladakhi Women
BMD-ultra distal radius, g/cm^2	0.47 ± 0.05	0.49 ± 0.06 (P = 0.29; E.S = 0.276)	0.41 ± 0.10
Osteopenia-ultra distal radius (n)	4	5	0
Osteoporosis-ultra distal radius (n)	0	0	4
BMD- calcaneus, g/cm^2	0.49 ± 0.08	0.49 ± 0.07 (P = 0.84; E.S = 0.528)	0.44 ± 0.10
Osteopenia- calcaneus (n)	5	6	4
Osteoporosis-calcaneus (n)	0	0	2

Values represented as Mean ± SD unless stated otherwise; SD, standard deviation; BMD, bone mineral density; E.S, effect size.

Table 3
25 vitamin D status of Sea Level Women and Ladakhi Women.

Group	Sufficiency (25 vitamin D > 30 ng/mL)	Insuffciency (25 vitamin D = 20–30 ng/mL)	Deficiency (25 vitamin D < 20 ng/mL)
Sea Level Women (%)	23	25	52
Ladakhi Women (%)	0	23	77

25 vitamin D levels were studied and classified according to the definition of World Medical Association (WMA)-adopted by the 66th general assembly in October 2015. 25 vitamin D, 25 hydroxy vitamin D.

Table 4
Serum concentrations of biochemical and endocrinal parameters.

Parameters	Sea Level Women Mean ± SD	Ladakhi Women Mean ± SD	P-value	Effect Size (Hedges' G)
Calcium, mg/dL	9.37 ± 0.98	9.87 ± 0.75	0.045*	0.706
Phosphorus, mg/dL	3.55 ± 0.75	3.34 ± 0.72	0.309	0.291
BAP (U/L)	20.40 ± 6.73	23.37 ± 4.42	0.131	0.505
25-OH vitamin D, ng/dL	20.86 ± 14.38	14.64 ± 6.79	0.076	0.542
iPTH, pg/mL	51.20 ± 21.78	37.99 ± 21.77	0.050*	0.607
Cacitonin, pg/mL	2.80 ± 2.118	6.89 ± 0.75	0.000**	2.505
Estradiol, pg/mL	114.73 ± 57.68	115.72 ± 76.27	0.961	0.015

Data presented as Mean ± SD unless stated otherwise. Significance of changes in mean values of Ladakhi and sea level women groups was determined by Student's *t*-test (**P < 0.01; *P < 0.05). SD, standard deviation; BAP, bone specific alkaline phosphatases; iPTH, intact parathyroid hormone.

was sufficient in 25 vitamin D while no LDK volunteer was sufficient in 25 vitamin D levels. Of these, 52% of SLN and 77% of LDK groups had 25 vitamin D deficiencies.

i-PTH and CT regulate bone resorption to maintain Ca levels in circulation. There was significant decrease (P = 0.051) in mean iPTH levels of LDK (37.99 ± 21.77 pg/mL) as compared to SLN volunteers (51.20 ± 21.78 pg/mL). CT levels also showed highly significant increase in LDK (P < 0.001) as compared to SLN. The mean CT levels were 6.89 ± 0.0.75 pg/mL and 2.80 ± 0.55 pg/mL in LDK and SLN groups, respectively. A low level of estradiol is responsible for post-menopausal osteoporosis. However, there was no change in mean estradiol level of LDK (115.72 ± 76.27) as compared to SLN subjects (114.73 ± 57.78).

4. Discussion

Osteoporosis, characterized by low bone mass and bone deterioration, is a major escalating health concern, with over 200 million people affected worldwide especially in developing countries due to increase in life expectancy and aging population [9]. In India, nearly 61 million people are suffering from osteoporosis of which 80% are women [10]. There are handful of studies on bone health of women from various parts of the country [11] but almost none from HA regions. Our study for the first-time reports bone health status in Ladakhi women by comparing them to age matched sea level counterparts.

The mean body height and weight of LDK in our study was observed to be less than SLN. These could be results of ethnic variation, poor nutritional availability, high energy expenditure in tough terrains of the region [12]. However, the BMI of both groups were similar and fell in normal range representing healthy volunteers in the present study. Therefore, reduced height and weight with healthy BMI in LDK group could be part of adaptive response of this population for survival at HA.

Reports including previous studies from our lab have consistently reported deteriorated bone density in low landers upon exposure to HA [5–8]. However, Bharadwaj et al [13] have reported higher bone mineral content in Ladakhi soldiers as compared to Tamilian soldiers using Allen's formula and reasoned it to wider bi-iliac, wrist, knee and ankle width of Ladakhi natives. But very recently, Zou et al [14] have reported high BMD, measured using DXA and low number of osteoporosis in the Tibetian population when compared to those from plains due to Ca rich diet comprising of meat and dairy products and sufficient light exposure, especially with routine outdoor pilgrimage. In the present study, we found similar BMD at both sites measured ie ultra-distal radius of non-dominant arm and left calcaneus in LDK and SLN. The mean BMD at the ultra-distal radius for Indian women as described by ICMR is 0.40 ± 0.04 which is lower than the mean BMD of both LDK and SLN subjects of our study [15]. These multi-centric studies throughout the country included women of all ages and socio-economic groups while our study groups mostly consisted of research fellows and staff at 2 institutes and therefore are having better BMD. Sudha et al. [16] have reported osteopenia and osteoporosis in the age group of 25–45 using quantitative ultrasound (QUS) at Jammu situated at 327 m and found 13% osteopenia and 7% osteoporosis at the calcaneus. In the present study, we found comparable cases of osteopenia in LDK and SLN groups at both sites measured in the study.

Bone is a highly dynamic tissue undergoing continuous remodeling with bone resorbing osteoclasts digesting the bone matrix and providing site for bone forming osteoblasts to lay new bone matrix [17]. Studies have also reported changes in biochemical and hormonal markers of bone remodeling in low landers upon exposure to HA [3]. BAP is major regulator of bone mineralization and hence major bone formation marker [18]. Nakamaru et al. [3] reported similar BAP levels after 10–20 years of mountaineering among male and female mountaineers. Our lab has previously shown decreased BAP levels in low lander soldiers exposed to both extreme and HA [7]. However, ALP levels were increased on exposure to HA but decreased in those deinducted from extreme altitude [6]. Ranhotra and Sharma [3] have reported increased ALP in high-lander of 1495 m, at Mizoram India due to increased bone turnover among high-landers. Increased ALP was also reported by Ramirez et al [19] in natives residing at 3000 m of southern Colombian Andes to sea level population. In the present study, we found insignificant changes in BAP levels of LDK and SLN subjects which indicate towards similar bone turnover in both groups.

Vitamin D is another important parameter with respect to bone formation as vitamin D is primarily responsible for Ca absorption required in bone mineralization [20]. According to the definition of World Medical Association (WMA) - adopted by the 66th general assembly in October 2015, 25-vitamin D levels below 20 ng/mL is considered vitamin D deficiency and levels between 20 and 30 ng/mL is considered vitamin D insufficiency. As reviewed by Aparna et al. [21] nearly 50–94% of healthy controls in community based Indian studies have vitamin D deficiency. Despite increased ultraviolet radiation at HA, most studies have reported low vitamin D among HA dwellers and sojourns [3]. Vitamin D deficiency was reported in high and moderate altitudes of Indian subcontinent at the Lahaul and Spiti valley located above 4000 m and Kashmir valley located at 1615 m, respectively [22, 23]. However, a recent study has reported natives of Tibet to have similar 25 vitamin D as compared to those from plains of China [14]. We did not find any difference in mean 25 vitamin D levels among LDK and SLN in our study but both groups presented prevalent vitamin D deficiency in the volunteers. Our study reports no LDK volunteers to be 25-vitamin D sufficient, which deficiencies were not limited to the LDK group and were reported in SLN as well. Low 25 vitamin D levels in LDK group could be due to dietary deficiency and reduced exposure to sunlight due to fully covered clothing in long winters of this region similar to Tibet [24].

Ca and P are fundamental elements of bone architecture. They form the inorganic part of the bone matrix called hydroxyapatite which constitute about 90% of bone matrix. Transient decrease in plasma levels of Ca was observed at HA which returned to normal after few days post Kanchenjunga expedition [25]. Similarly, no change in serum Ca was observed by Kasprzak et al. in alpinist climbers at 3200–3600 m. However, a significant decrease in plasma ionized Ca and phosphate levels were reported on exposure to HA of 4424 m for 5 days as reported by Khan et al. [3]. Similarly Tanaka et al. [5] reported decreased serum Ca along with increased serum P, urinary P and urinary Ca levels during Himalayan expedition at HA of 3700 m for 60 days and extreme altitude of 5400 m for 37 days. Our lab previously reported increased serum Ca levels in soldiers posted to HA as compared to those at sea level [7]. There was, however, no change in serum Ca levels of soldiers de-inducted from extreme altitude [6]. In another study involving 8 women exposed to 4300 m for 65 days, the authors reported increased serum Ca which did not recover even after 2 weeks stay at sea level. However, in the same study phosphate levels increased initially but reverted back to normal levels after a week [26]. Basu et al [6] found increased P levels in those de-inducted from extreme altitude. The varied results of Ca and P levels among HA sojourns could be due to difference in diet followed during each expedition or stays at HA. Soldiers and mountaineers usually maintain their diet at HA through a ration system while natives depend upon local availability of food sources, affected by rocky terrain-leading to reduced agriculture and transport facilities in HA regions [27]. In our study, LDK had significantly increased serum Ca while non-significant decreased in serum P levels. High serum Ca levels are indicative of increased bone resorption, releasing Ca into circulation [28] but because we did not find any difference in BMD or cases of osteopenia in the 2 groups, another plausible reason could be increased Ca in the diet of LDK population consisting of vegetation grown on rocky terrain, milk and meat products similar to Tibetans [14]. Ca and P have signaling and structural roles in the body; therefore, their levels need to be maintained within normal range for proper physiological functions. Both minerals were within normal range in SLN and LDK subjects of our study.

i-PTH and CT hormones are involved in maintaining serum Ca and P levels in the body and have antagonistic roles [29]. Parathyroid hormone (PTH) is released from parathyroid gland and increases Ca by increasing bone resorption via increased osteoclastogenesis in response to low serum Ca. CT is released from the thyroid gland and reduces Ca levels by increasing bone mineralization in response to high serum Ca [30]. Kasprzak et al reported no change in serum PTH levels of alpinist climbers at 3200–3600 m. However, Khan et al.[3] reported increased PTH values in low landers exposed to altitude of 4424 m for 48 h which decreased after 96 h. Tanaka et al. [5] reported decreased PTH value after expedition at both high and extreme altitude for 60 and 37 days, respectively. Our lab previously reported increased PTH levels after de-induction from extreme altitude and decreased PTH values when soldiers posted at HA were compared with sea level counterparts [6,7]. There was significant decrease in i-PTH levels in LDK group as compared to SLN in our study which may have relation to increased Ca levels in this group. Apart from our lab only Tanaka et al. have reported CT levels after exposure to HA. All 3 studies have reported decrease in serum CT levels after exposure to high and extreme altitudes [5–7]. However, in the present study, CT levels were significantly high in LDK subjects which may again be due to increased serum Ca and may be playing a role in Ca homeostasis by functioning in opposition to PTH.

Nitric oxide (NO) through nitrite levels has been implicated in various processes involved in adaptation of Tibetian highlanders compared to low landers ascending to HA [31]. Our laboratory has recently reported higher levels of nitrite and nitrate in Ladakhi women pointing towards higher endothelial NO production in this population [3]. NO in optimum range has been reported to avoid osteoclast mediated bone resorption by not allowing attachment of osteoclast to bone matrix [32] while promote osteoblast proliferation, differentiation and survival thus promoting bone formation [33]. Nascimento et al [33] have reviewed the role of NO in osteogenesis quite extensively in the article titled “How can nitric oxide help osteogenesis?”.

Estrogen is reported to improve NO bioavailability by post translational modification of eNOS [34]. Post-menopausal women have more osteoporosis and osteoporotic facture due to lack of estrogen and therefore, increase in iron accumulation [35]. Hormone replacement therapy for estrogen deficiency is widely used to treat postmenopausal osteoporosis [36]. In the present study, we also observed equivalent estradiol levels in LDK and SLN subjects. From the above observations it may be concluded that Ladakhi women have enough estrogen to prevent bone deterioration. Estrogen could also be aiding bone formation in Ladakhi women to maintain bone health by inhibiting sclerostin, an inhibitor of Wnt pathway involved in bone formation upon mechanical loading [37]. Post-menopausal LDK volunteers had reduced BMD and exhibited cases of osteoporosis further pointing the protective role of estrogen in premenopausal LDK volunteers.

Challenges such as hypobaric hypoxia, cold and dehydration prevalent at HA have been reported to exert its effect on bone health individually. Hypobaric hypoxia has been reported to deteriorate bone quality in animal models by several authors [3]. On the other hand the effect of cold and dehydration on bone density has been inconclusive [38–40]. Dietary habits and physical activity are other factors contributing to bone density and may be affecting bone health of subjects in our study as well. Almost all subjects of our study had a sedentary lifestyle [41]. However, the tough terrain of Ladakh consisting of steep, rocky hills and lack of transport facility in HA areas [42] may result in increased physical activity and therefore increased bone formation due to mechanical loading through Wnt pathway [43].

The findings of this study must be considered in the context of several limitations. First, the sample size is very small. Second, only 2 sites were measured for bone density whereas whole body DXA such as in clinical settings would have been a better indicator of bone density status but a lighter machine for field studies was required for screening volunteers in tough terrains of HA. Despite these limitations, our study for the first time reports bone health status of Ladakhi women by accessing bone density measurement using DXA, biochemical and hormonal indicators. This pilot study sample therefore adds to sparse literature on bone health parameters of a specific ethnic group residing at HA.

5. Conclusions

The observations of this cross-sectional study suggest no significant relationship between high altitude living and BMD among Ladakhi women, staying above 3500 m for generations. These women seem to have maintained bone mineral health in-spite of harsh environment of high altitude as significant changes were not observed in BMD measurements among the 2 groups at either site measured in this study. Both groups of the study were reported to have equal prevalence of osteopenia which could be related to sedentary lifestyle and nutritional deprivation. Except Ca, i-PTH and CT, all other biochemical and endocrinal parameters were similar among the 2 groups studied. However, both groups also exhibited high prevalence of 25 vitamin D deficiencies which could be reason for osteopenia observed in both groups. NO and estrogen along with rocky terrain of the region influencing micronutrients and physical activity could play a role in maintaining bone health in Ladakhi women from hypobaric hypoxia mediated bone loss. Bone mineral metabolism is a complex process regulated by various pathways and factors which may be different for HA native and sojourns leading to different observations. Detailed studies elucidating cellular and molecular pathways can improve our understanding of HA adaptation with respect to bone mineral metabolism. Both groups exhibited vitamin D deficiency and therefore appropriate preventive measures such as vitamin D supplementation may reduce the prevalence osteopenia in the studied populations.

CRediT author statement

Lijy K Babu: Investigation, Data curation, Formal analysis, Writing - Original Draft. **Snigdha Shaw:** Investigation. **Dishari Ghosh:** Conceptualization, Methodology, Funding acquisition, Supervision, Writing - Review & Editing.

Conflicts of interest

The authors declare no competing interests.

Acknowledgments

We express our gratitude to Defence Research and Development Organization (DRDO) for funding of the project. Authors are highly obliged to all study participants from DIPAS, Delhi, and DIHAR, Leh, India. We are especially grateful to Dr. Bhuvnesh Kumar (former Director, DIHAR and DIPAS) for his constant support. Finally, we appreciate the technical support of Dr. Niroj Sethy, Dr. Kaushik Ray and Mr. Mukesh Chandra. Without their assistance, the study would not have been possible. **ORCID** Lijy K Babu: 0009-0003-4818-4785. Snigdha Shaw: 0009-0000-1964-5253. Dishari Ghosh: 0000-0002-1300-6538.

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