



# Looking at Mountains: Role of Sustained Hypoxia in Regulating Bone Mineral Homeostasis in Relation to Wnt Pathway and Estrogen

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## Abstract

Deteriorated bone microarchitecture is a major health concern affecting millions worldwide, amounting to high mortality along with psychological, social, and economic burden. Hypoxia has been known to affect bone mineral metabolism in various in vitro and in vivo experiments in an inconclusive manner and only a few studies are available on natives or travelers of high altitude, pointing towards the deterioration of bone health. HIF proteins, fundamental to hypoxia signaling have also been shown to affect bone remodeling by mediating osteoblastogenesis and osteoclastogenesis but the underlying mechanism of this process is not clear. Most studies have been reported in men but only few in female, while it has been already established that estrogen plays a major role in protecting skeletal health and recent reports identify estrogen as a major player in determining bone quality in men as well. The tough terrain and lack of transport in these areas require optimal bone quality to be maintained for continuous locomotion and load-bearing capacity. The Wnt pathway is involved in load-induced bone formation and sclerostin; the inhibitor of this pathway has been reported to be regulated by both estrogen and HIF proteins. However, the hypobaric hypoxia-operated molecular mechanism regulating the bone quality and microarchitecture in both male and female is still not fully elucidated. Therefore, in this review, available literature on the bone health status under sustained hypoxic exposure focusing on the significance and crosstalk of HIF proteins, Wnt pathway, and estrogen are compiled and discussed to open new aspects of high-altitude bone health research.

**Keywords** Bone remodeling · Estrogen · HIF · Hypoxia · Osteoporosis · Wnt pathway

## Introduction

Osteoporosis, characterized by low bone mass and bone deterioration, is a major escalating health concern with about 200 million people being affected worldwide [1]. The local microenvironment is an important determinant of skeletal cell functions. A small change in inspired or delivered oxygen (O<sub>2</sub>) influences skeletal cell homeostasis, i.e., the balance between bone formation and bone resorption [2]. Pathological and environmental hypoxia has been reported to influence bone health [3–6]. Approximately 140 million people live at

elevations equal to or above 2500 m defined as high altitude (HA) while another 40 million people are visiting HA every year for recreational and occupational purposes [7, 8]. A range of physiological adjustments are required for survival at HA due to extreme environmental conditions like cold, dehydration, solar radiation, malnutrition, and hypobaric hypoxia [9]. With the increase in altitude, there is a decrease in barometric pressure resulting in fewer O<sub>2</sub> molecules in the inspired air and subsequently organ tissues, causing hypoxia [10]. Fortunately, acclimatization allows partial compensation for lowered O<sub>2</sub> levels and the body responds to hypobaric hypoxia via. molecular, cellular, and systemic adaptations across cardiovascular, respiratory, musculoskeletal, hormonal, reproductive, and metabolic organ systems [11–17]. At the molecular level, adaptation to hypoxia is driven majorly by the hypoxia-inducible factor (HIF) family of transcription factors [18]. Skeletal cells also respond to hypoxia via hypoxia signaling pathway consisting of HIF proteins and reports have highlighted the importance of HIF proteins in regulating several steps during growth, development, and

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repair in all skeletal cell types viz. chondrocytes, osteoblasts, osteocytes, and osteoclasts [19].

Hypoxia has also been reported to alter various pathways like vascular endothelial growth factor (VEGF) bone morphogenetic proteins (BMP) and Wnt pathway involved in the maintenance of bone mineral homeostasis [20, 21]. Bone health parameters like vitamin D (Vit D), calcium, phosphorus, alkaline phosphatases (ALP), and hormones like sex steroid hormones, intact parathyroid hormone (iPTH), calcitonin, etc. are also affected by hypoxic exposure [22–24]. The great Himalayas are a major geographical feature of the Indian sub-continent and a large population inhabits these altitudes. Almost no study has reported the epidemiology of osteoporosis among natives of these altitudes, but two studies have reported osteoporosis and a significant percentage of osteopenia after exposure to the HA environment [22, 24]. Bone microarchitecture and bone mineral density (BMD) have a profound effect on bone strength and load-carrying capacity, [25] required for continuous movement on tough terrains of HA [26]. Literature exploring the field of bone health at HA is scanty and most of the available studies have been reported in males. Females constitute nearly half the demography of any geographical area and also a large number of women are visiting these altitudes nowadays for professional and recreational purposes. Participation of women in the Indian army, particularly posted at HA has also increased over the last decade. We are committed to studying the physiology of Indian soldiers posted at HA and stress like hypobaric hypoxia has been reported to deteriorate bone health at these altitudes. We have, therefore, reviewed available literature describing the role of sustained hypoxia in regulating bone health in relation to Wnt pathway and estrogen at HA.

## Bone Mineral Metabolism and Incidences of Osteoporosis in India

Bone is often misunderstood as merely an organ that supports the body structurally, but it has many other functions like protecting our internal organs from injury, enabling us to move, serving as a reservoir of minerals like phosphorus and calcium, and providing a suitable environment for hematopoiesis [27–29]. Most bones grow in length through endochondral ossification where primary spongiosa is created by bone-eating osteoclasts which mediate resorption of calcified cartilage, finally replaced by bone-forming osteoblasts which then deposit the bone matrix [30, 31]. Despite its static and rigid impression, bone is continuously remodeled where old bone tissue is digested by osteoclasts, and new bone tissue is laid down by osteoblasts in a tightly coordinated manner to ensure same rate of resorption and formation, thus maintaining skeletal integrity [27]. Bone

remodeling allows for the adaptation of the skeleton to meet changing mechanical needs and homeostasis of minerals like phosphorus and calcium [28]. Bone remodeling is regulated by systemic regulators, including hormones (e.g., parathyroid, calcitonin, growth, and sex hormones), glucocorticoids, prostaglandins, and local regulators including many cytokines and growth factors [27, 32–37]. Slight alterations in the optimal values of the above mentioned regulators may disturb the bone homeostasis leading to sub-optimal quality. WHO has stratified the following definitions of 4 categories of bone health based on standard deviations (SDs) below the mean peak bone mass of young healthy adults as determined by DEXA, with BMD at the femoral neck as the reference site: Normal: a BMD value of 1 SD below, Osteopenia: a BMD value between 1 and 2.5 SD below, Osteoporosis: a BMD value > 2.5 SD below, and Severe (established) Osteoporosis: a BMD value > 2.5 SD below with one or more fragility fractures [38, 39].

The prevalence of osteoporosis is becoming a major public health issue due to the universal increase in life expectancy; in particular, more rapidly in the developing countries of Asia, South America, and Africa [38]. In India, there are around 50 million individuals with osteoporosis or osteopenia [40, 41]. The Indian Council for Medical Research (ICMR) in its attempt to generate an India-specific database showed that Indians have lower BMD than their North American counterparts [42]. Several factors like a predominant vegetarian diet containing high amounts of phytates, oxalates, and fiber, low consumption of milk and milk products, negligible intake of calcium and Vit D supplements, reduced sunlight exposure, lack of physical activity, increased glucocorticoid consumption, genetic differences, and poor level of awareness contribute to osteoporosis in India [43, 44]. A study reported a high prevalence of osteopenia (52%) and osteoporosis (29%) among Indian women aged 30–60 years of low-income groups which may be attributed to inadequate nutrition [45]. Another study observed that osteoporosis at the spine and hip when computed using the Hologic database was present in 42.7% and 11.4% of subjects respectively but only in 27.7% and 8.3% of subjects using the ICMR database [46]. The decrease in bone mass and microarchitectural deterioration of bone tissue also result in an increased risk of fracture and therefore almost 40% of women and 20% of men with osteoporosis have been reported to have at least one osteoporotic fracture during their lifespan [47, 48]. Reports suggest that more than 4.5 million women in India above the age of 60 years have fractured spine and approximately 0.25 million people sustain a hip fracture every year due to osteoporosis [49]. The annual incidence rate of hip fractures above the age of 55 years has been reported to be 163 and 121 per 100,000 people per year for women and men, respectively [44]. Initially, it was thought that women are more predisposed to osteoporosis, but reports have indicated it to

be a major health concern among males as well [50–52]. A significant percentage of men in various studies showed compromised bone quality [44, 53, 54]. A study even reported earlier age of fracture in men as compared to women in north India [54].

## Bone Health Status at HA

To understand the effect of hypobaric hypoxia on bone health, it is important to look at the bone health status of natives of HA, living in these regions for generations and therefore, may represent long-term effect of hypobaric hypoxia exposure on skeletal health. High-altitude natives (HANs) have been reported to have short stature, fracture-prone thin metacarpals, and enlarged medullary cavity in long bones [55]. An enlarged medullary cavity could be an adaptation for increased erythropoiesis in order to compensate for low O<sub>2</sub> [55]. A study showed that Vit D was very low, and bone-related diseases were significantly higher in Tibet [56]. Another study which compared ALP activity in dwellers of moderately HA at 1495 m and low altitude of 250 m in Meghalaya, India, reported increased ALP activity among HANs which may be due to the elevated rate of bone turnover at these altitudes [57].

Few short-term hypobaric hypoxia exposure studies have also been reported where the bone health of the participants was evaluated. When BMD was assessed in 24 members of the Himalayan expedition party who stayed at 3700 m for 60 days and 5400 m for 37 days, a decrease in BMD of the radius was observed which recovered partially in 12 months post expedition [3]. The Indian armed force composed of healthy males who stayed at 3450 m for about 4 months demonstrated a decrease in bone health measured by speed of sound (SOS) values and *T* scores of the radius, metatarsal, and phalanx, with a higher percentage of subjects having osteopenia and osteoporosis compared to their counterparts at sea level [22]. Another study by the same authors at extreme HA of 5400–6700 m reported similar results and 62% of these subjects had osteopenia while 2.8% had osteoporosis after de-induction [24]. Likewise, a group of five healthy, active male adults, who participated in an expedition of 24 weeks at 2500 m in Antarctica, showed a decrease in BMD-spine [58]. Another group that studied bone metabolism in middle-aged and older mountaineers and compared them with those who walk regularly and those who do not exercise regularly showed no significant difference in osteo sono-assessment index of the right calcaneus as an indicator of bone strength among the three groups [59]. However, it is equally interesting that the study on Ladakhi soldiers, native to altitudes greater than 3500 m showed decreased bone mineral content after a stay of 44 days at

sea level [60]. In summary, several authors have shown bone health to be deteriorating at HA.

## Changes in Bone Remodeling Factors at HA

Residency at HA changes different hormonal, biochemical, and morphological indicators of bone remodeling. A significant decrease in plasma ionized calcium and phosphate levels with fluctuating PTH concentration was reported on exposure to HA of 4424 m for 5 days [61]. Similarly decreased serum calcium, calcitonin, 25-Vit D, C-PTH with increased serum and urinary phosphorus along with urinary calcium levels was reported post-Himalayan expedition at HA of 3700 m for 60 days and extreme altitude of 5400 m for 37 days [3]. Bone formation and resorption markers were measured in healthy individuals after a stay at different high to extreme altitudes [22, 24] and it was observed that 40 weeks stay at an extreme altitude of 5400–6700 m led to decreased bone health associated with decreased bone formation markers viz. ALP, bone-specific alkaline phosphatase (BAP), and calcitonin indicating decreased bone formation [24]. Calcium levels were maintained at extreme altitudes [24]. At HA, BAP, urinary deoxypyridinole to creatine ratio (DPD/Cr), C-terminal collagen propeptide (CICP), collagen type 1 cross-linked N-telopeptide (NTX) were reported to be lower than sea level reflecting a lower bone turnover but ALP and calcium levels increased [22]. Serum 25-Vit D showed significant reduction at both extreme and HA [22, 24]. 2-week expedition at 3200–3616 m caused a significant decrease in 25-Vit D levels with a significant increase osteoprotegerin (OPG) levels but soluble receptor activator of NF- $\kappa$ B ligand (sRANKL) levels showed no changes after the expedition [62]. A significant decrease in the 25-Vit D concentration in the alpinist climbers after their return from the mountaineering expedition with no changes in serum PTH and calcium was also reported [63]. iPTH is a regulator of calcium metabolism and aids the production of osteoclast which speeds up bone resorption, releasing calcium and other minerals into circulation [64, 65]. Plasma osteopontin (OPN) levels were reduced after exposure at 3500 m for 1 day in healthy adults [66]. One group reported that 10 to 20 years of mountaineering lead to non-significant changes in BAP, procollagen type 1 amino-terminal propeptide (P1NP) and bone formation/resorption ratio in male and female mountaineers with significantly higher tartrate-resistant acid phosphatase-5b (TRACP-5b) levels [59]. Table 1 summarizes studies done on travelers of HA to understand the effect of hypobaric hypoxia on bone metabolism. Studies report significant changes in biochemical and endocrinal factors related to bone mineral metabolism. However, it is

**Table 1** Studies on the effect of short-term hypobaric hypoxia exposure to bone health parameters of travelers

References	Sample description			Outcome
	Type	Altitude	Duration	
O'Brien et al. [58]	Healthy adults (24–58 years) <i>n</i> = 5	2500 m	24 weeks	↓BMD-spine
Tang et al. [66]	Healthy adults (> 17 years) <i>n</i> = 278	3500 m	1 day	↓ OPN
Sliwicka et al. [62]	Healthy adults (23–31 years) <i>n</i> = 8	3200–3616 m	2 weeks	↓25 Vit D ↑OPG #sRANKL
Kasprzak et al. [63]	Healthy adults (23–37 years) <i>n</i> = 9	3200–3616 m	2 weeks	↓25 Vit D #Calcium #PTH
Basu et al. [22]	Healthy adults (21–47 years) <i>n</i> = 2600	3450 m	16 weeks	↓SOS-R #SOS-T ↓SOS-M ↓SOS-P ↓T score-R ↓T score-P ↑ALP ↓BAP ↑Calcium deposit ↓25-Vit D ↓i-PTH ↓Calcitonin ↓CICP ↓NTX ↓DPD/Cr
Basu et al. [24]	Healthy adults (21–47 years) <i>n</i> = 221	3000–3754 m 5400–6700 m	24 weeks 16 weeks	#SOS-R #SOS-T ↓SOS-M ↓SOS-P #Z score-R #Z score-T ↓Z score-M ↓Z score-P #Calcium ↑Phosphorous ↓ALP ↓BAP ↓25-VitD ↓Calcitonin ↑i-PTH #DPD/Cr
Khan et al. [61]	Healthy adults (18–34 years) <i>n</i> = 20	4424 m	5 weeks	↓Calcium ↓Phosphorus
Tanaka et al. [3]	Healthy adult (24–44 years)	3700 m 5400 m	60 days 37 days	↓Bone density ↓Serum-cPTH ↓Serum-25-Vit D ↓Serum-calcitonin ↓Serum-calcium ↑Serum-phosphorus ↑Urinary-calcium ↑Urinary-phosphorus
Bharadwaj et al. [60]	Healthy adult Natives <i>n</i> = 48	3960–4270 m	44 weeks	↓Bone mineral (kg)

↑, upregulated, ↓ downregulated, # unaltered, NR not reported, R one-third of distal radius, P proximal third phalanx, M fifth metatarsal, T mid-shaft tibia

difficult to conclude the effect of HA on bone health based on these factors and more studies are required in the field to come to conclusion.

## Effect of Hypoxia on Bone Remodeling Parameters In Vitro and In Vivo

To understand the effect of hypobaric hypoxia on bone mineral homeostasis we looked into the existing literature on the gene and protein expression of various markers of bone remodeling under the effect of sustained chronic hypoxia during osteogenic differentiation. Runt-related transcription factor 2 (RUNX2) is a transcription factor that regulates the expression of osteogenic differentiation-related genes like ALP, type I collagen (COL1A1), OPN, and osteocalcin (OCN) [67, 68]. While most studies showed a decrease in RUNX2 gene and protein expression when exposed to 1–2% hypoxia for 5–21 days [69–79], results are not conclusive as expression was maintained in few studies and even increased after 2–5 days of exposure to chronic hypoxia at 1.5–5% O<sub>2</sub> [72, 80–82]. Protocols involving 1–2% O<sub>2</sub> for 5–21 days reported a decrease in ALP gene expression [73, 74, 76], while 2–5% O<sub>2</sub> for 3–14 days showed increased expression [67, 72, 80, 83]. ALP protein expression was also found to be increased when less severe hypoxia of > 2% O<sub>2</sub> for 2–21 days was applied [84–87] while ALP activity was decreased after severe hypoxic exposure of 1–2% O<sub>2</sub> for 2–28 days [68–70, 73, 75–78, 88]. Col1A1 also showed contradictory results as the expression of the Col1A1 gene was unaltered at 2 and 5% O<sub>2</sub>; it decreased after exposure to 2% O<sub>2</sub> for 7, 14, or 21 days, and increased with 2% O<sub>2</sub> for 12 days and 3% O<sub>2</sub> for 3 days with a decrease after 9 days, indicating a decreased osteogenic potential [67, 71–73, 76, 83, 89, 90]. A lower gene expression of OPN was observed following chronic hypoxic exposure of 1–2% O<sub>2</sub> for 21 days, but expression was maintained at 5% [70, 74, 78, 91–93]. Lower expression of the OCN gene was reported when severe hypoxic exposure of 5–28 days were applied while four studies showed increased expression with a moderate dose of > 2% O<sub>2</sub> for a short exposure time of 3–12 days [67, 72, 74, 76, 80, 83, 91–94]. Further, when calcium depositions were investigated, no change in calcium deposition was found after exposure to 1–5% O<sub>2</sub> for 14–21 days, while a severe dose of < 2% O<sub>2</sub> resulted in decreased calcium deposits [67, 70, 74, 76, 88, 91, 93, 95–97]. Table 2 summarizes various in vitro studies included in reviewing the effect of sustained hypoxia on various bone parameters. Based on the above discussions, it may be concluded that chronic hypoxic exposure of 1–2% O<sub>2</sub> with extended exposure time resulted in bone resorption. The results of in vitro studies on osteogenic differentiation are difficult to conclude as there are differences in observations which may have resulted due to differences in the amount and

duration of hypoxia used in the experiments. Also, the different cell lines used may add to variations observed.

The response of cultured cell lines to normobaric hypoxia may not give full insight into the physiological response to hypoxia especially hypobaric hypoxia because at the systemic level, the body tries to compensate for low O<sub>2</sub> availability by physiological adjustments like increased erythropoiesis, heart rate, and ventilation eventually reaching sufficient O<sub>2</sub> at the tissue level. Therefore, we looked into various in vivo studies to evaluate the effect of systemic hypoxia on bone health in mice and rat models. Table 3 summarizes various animal studies on rat or mouse models included in this review. Healthy rats exposed to simulated hypoxia at 6000 m for 3 weeks, showed a decrement in the bone volume to tissue volume ratio (BV/TV), tubercular number (Tb.N), and BMD-total [98]. In another study where rats were exposed to hypobaric hypoxia equivalent to 4100 m for varied periods of 2, 4, 6, or 8 months, a decrease in femur length, femur dry weight, cortical area, load fracture, bone stiffness, and second moment of inertia of cortical bone (CSMI) were observed [99]. Similar results of reduced bone mineral content, bone strength, and elasticity were observed

**Table 2** In vitro studies of the effect of hypoxia on bone metabolism

Reference	Oxygen level	Duration	Results
Huang et al. [76]	0.1%	1 day	↑RUNX2-gene
Gao et al. [84]	1%	21 days	↑RUNX2
Ding et al. [74]		21 days	↑ALP-gene ↑ALP
Lambertini et al. [82]	1.5%	2 days	↑ALP-activity
Lee and Kemp [87]	2%	NR	↑Col1A1-gene
Zhang et al. [68]		2 days	↑Col1A1
Salamanna et al. [89]		3 days	↑OCN-gene
Ciapetti et al. [67]		12 days	↑OCN
Tsang et al. [252]		14 days	↑Calcium deposit
Tsang et al. [252]		21 days	
Xu et al. [72]	3%	3 days	
Balogh et al. [80]	5%	NR	
Gu et al. [83]		3 days	
Buravkova et al. [81]		14 days	
Ding et al. [74]		14 days	
Sengupta et al. [253]		49 days	
Binder et al. [95]	1%	21 days	#RUNX2-gene
Ding et al. [74]		21 days	#ALP-gene
Zhao et al. [71]	2%	NR	#ALP
Xu et al. [69]		5 days	#ALP-activity
Xu et al. [69]		8 days	#Col1A1-gene
Ciapetti et al. [67]		12 days	#Col1A1
Tsang et al. [252]		12 days	#OCN
Zhang et al. [68]		14 days	#OPN-gene
Tsang et al. [252]		14 days	#OPN
Lee et al. [91]		21 days	#Calcium deposit
Holzwarth et al. [254]	3%	14 days	
Burakova et al. [81]	5%	NR	
Russo et al. [78]		3 days	
Gu et al. [83]		21 days	
Binder et al. [95]		49 days	
Sengupta et al. [253]			

**Table 2** (continued)

Reference	Oxygen level	Duration	Results
Lee et al. [79]	1%	NR	↓RUNX2-gene
Hsu et al. [92]		NR	↓RUNX2
Park et al. [93]		NR	↓ALP-gene
Ma et al. [77]		2 days	↓ALP
Ding et al. [74]		2 days	↓ALP-activity
Yao et al. [255]		2 days	↓Col1A1-gene
Xu et al. [70]		21 days	↓Col1A1
Yang et al. [97]		21 days	↓OCN-gene
Cicione et al. [96]		21 days	↓ OCN
Zhao et al. [71]	2%	NR	↓OPN-gene
Burian et al. [256]		NR	↓OPN
Salamanna et al. [89]		3 days	↓Calcium deposit
Xu et al. [69]		5 days	
Huang et al. [76]		5 days	
Pattappa et al. [88]		6 days	
Xu et al. [69]		8 days	
Zhang et al. [68]		14 days	
Huang et al. [76]		14 days	
Chen et al. [207]		14 days	
Tsang et al. [252]		21 days	
Xu et al. [69]		21 days	
Lee et al. [91]		21 days	
Chen [207]		21 days	
Xu et al. [72]	3%	9 days	
Holzwarth et al. [254]		14 days	
Russo et al. [78]	5%	NR	
Pattappa et al. [88]		6 days	
Inagaki et al. [85]		14 days	
Merceron et al. [75]		28 days	

↑, upregulated, ↓, downregulated, #, unaltered, NR not reported

in other studies of stimulated hypobaric hypoxia exposure at 2000–5500 m [100–104]. Alterations in genetic and protein expression of various biomarkers were also reported in rats and mice after hypobaric hypoxia exposure. Rats exposed to 4000 m for 3 weeks showed a decrease in RUNX2 and OCN protein levels while no change in levels of ALP was observed which may be due to exposure to the lesser altitude as other parameters of bone strength like BMD, bone volume (BV), BV/TV, trabecular thickness (Tb.Th), and Tb.N did not alter the experiment [105]. In another study where mice were exposed to 19,000 m for 4 days, no change was observed in BV/TV, but increased numbers of osteoclasts along with decreased numbers of osteoblasts and colony-forming unit (CFU) were reported [106]. Overall major in vivo studies are pointing towards deteriorated bone quality when animals were exposed to stimulated hypobaric hypoxia.

## Oxygen Sensing in Bone

Bone has a highly hypoxic microenvironment with partial pressure of oxygen ( $pO_2$ ) ranging from < 1% in highly hypoxic regions to 6% in sinusoidal cavities [107]. Hypoxia in bone is likely a result of successive

drops in velocity and oxygenation in sinusoidal blood and enormous  $O_2$  consumption by hematopoietic cells [108–110]. Prolyl hydroxylase (PHD) enzymes (PHD1, PHD2, and PHD3) are the primary cellular oxygen sensors [111]. Along with HIF proteins, PHD enzymes are fundamental for any cell to adapt and survive in hypoxic situation. In well-oxygenated tissues, proline residues of HIF- $\alpha$  are hydroxylated by PHD which provides a binding site for von Hippel–Lindau tumor-suppressor protein (VHL), initiating proteolytic degradation of HIF- $\alpha$ . When  $O_2$  levels are limited, PHD-dependent hydroxylation is reduced allowing HIF- $\alpha$  to dimerize with HIF- $\beta$  and binds to hypoxia response elements (HRE) in the nucleus [112, 113]. HIF signaling influences cell and tissue functions at multiple levels to reverse the decreased  $O_2$  tension by stimulating angiogenesis and manipulating cellular metabolism [114]. HIF signaling induces transcription of various genes of bone remodeling such as VEGF production in osteogenic cells attracts blood vessels for the supply of oxygen, nutrient, osteogenic factors, and possibly osteoprogenitors, and thereby stimulating bone formation [114]. HIF-signaling also drives glycolysis in osteoprogenitor cells and controls bone homeostasis by regulating EPO, RUNX2, OPN, OCN, ALP, and sclerostin (SOST) secretion in bone cells [114].

## Bone Remodeling Under Hypoxia Condition

Bone remodeling maintains the skeletal integrity by maintaining a balance between bone formation and bone resorption [19]. Osteoblasts are bone-forming cells and the process of maturation of mesenchymal stem cells into mature osteoblasts is known as osteoblastogenesis [115]. Osteocytes are formed when osteoblasts get buried in the bone matrix and differentiate to develop a dendritic process that extends into the bone marrow and bone surface for the transfer of nutrients and gases as well as communication [116]. Osteoclasts are bone-degrading skeletal cells and formation of mature osteoclasts from monocytes is known as osteoclastogenesis [117]. At puberty when the body is trying to reach its peak bone mass there is an increase in bone formation improving bone mass and quality while there is an increase in bone resorption in old age deteriorating bone [118, 119]. The smallest changes in the bone microenvironment such as hypoxia can disturb this homeostasis between osteoblastogenesis and osteoclastogenesis which is a dynamic process known as bone remodeling [2].

## Hypoxia and Bone Formation

Hypoxia has been reported to limit bone formation by delaying osteoblast growth differentiation and bone mineralization [120–122]. During an in vitro experiment, the formation

of mineralized bone nodules decreased by 11-fold following exposure to 2% O<sub>2</sub> while it almost stopped at 0.2% O<sub>2</sub>. This effect was partly due to decreased osteoblast proliferation [123]. Hypoxia is also known to reduce ALP activity, and OPN, OCN, and RUNX2 expression in osteoblast cell lines [124, 125]. In ovariectomized rats, short-term hypoxic exposure was shown to suppress osteoblastogenesis [126] which may be linked to reduced RUNX2 expression, which in turn reduced the differentiation of mesenchymal cells to immature osteoblasts [127–130]. Limited expression and activity of ALP as well as collagen in hypoxic environments resulted in the inhibition of osteoblast proliferation. Also, the collagen produced had a lower amount of hydroxyl residue leading to reduced matrix mineralization [123]. Inhibition of osteoblast function by hypoxia could also be the result of reduced activity of proline hydrolases and/or lysyl oxidase which are O<sub>2</sub>-dependent enzymes [123, 131] and required for post-translational modification of collagen [123, 132]. Hypoxia has also been reported to increase SRY box 9 (SOX9) levels, a negative regulator of osteoblastogenesis through decrease in RUNX2 levels [71, 80]. Hypoxia-mediated apoptosis due to mitochondrial dysfunction and mitophagy may be another reason of bone loss upon exposure [133]. The osteogenic differentiation and expression of VEGF reached its peak after 3 days of hypoxia implying that the duration of hypoxia is an important factor for the differentiation of precursor cells [134].

HIF-1 $\alpha$  overexpression significantly stimulated osteoblast cell viability, which was suppressed by silencing HIF-1 $\alpha$  and led to reduced expression of osteogenic markers, including RUNX2, ALP, and OCN [135]. Mice overexpressing HIF-1 $\alpha$  showed increased vascularity and bone formation, whereas mice lacking HIF-1 $\alpha$  had reduced repair efficiency [136]. The improved bone quality in ovariectomized mice after PHD inhibition could be linked to increased number of osteoblasts in mice overexpressing HIF-1 $\alpha$  compared to controls [137, 138]. HIF-1 $\alpha$ -driven bone formation may act via upregulation of glycolytic activity as VHL deletion in osteoblasts boosted cellular glycolysis [139, 140]. Hypoxic exposure may therefore increase HIF-1 $\alpha$ -induced VEGF transcription, improving local vascularization which in turn might increase nutrient availability for increased cellular glycolysis and activation of target genes in progenitor cells finally enhancing bone formation through osteogenic–angiogenic coupling [20, 136, 138–142]. The osteoanabolic response of HIF-1 $\alpha$  stimulation could also be the result of osteogenic–angiogenesis through VEGF, aiding in reaching sufficient O<sub>2</sub> levels. VEGF may not be able to yield the same anabolic response in the absence of O<sub>2</sub> in *in vitro* systems [123]. HIF-2 $\alpha$  which was initially thought to have no impact on osteoblast has also been implicated in osteoblastogenesis [142–145]. Reduced HIF-2 $\alpha$  expression promoted osteoblast differentiation leading to increased

bone mass [143, 144]. HIF-2  $\alpha$  promoted SOX9 through a reduction in the expressions of RUNX2 and SP7 which are osteoblast differentiation mediators [144, 146]. In hypoxic environment with increased HIF (both 1 $\alpha$  and 2 $\alpha$ ) stabilization, there is an increase in TWIST expression leading to reduction in RUNX2 and OCN expressions resulting in decreased osteoblast mineralization and bone mass [127–130, 143, 147]. Major studies have reported hypoxia to be inhibiting osteoblast number and function in *in vitro* systems however, HIF-1 $\alpha$  has been reported to increase osteoblastogenesis through osteoanabolic response of VEGF in *in vivo* models. At the same time, two new studies have reported HIF-2 $\alpha$  impairing osteoblastogenesis.

### Hypoxia and Bone Resorption

Hypoxia was shown to be a major stimulator of osteoclast formation and bone resorption as a fourfold increase in osteoclast number and 21-fold increase in its activity was reported after exposure to 2% O<sub>2</sub> [123, 148–150]. A reduction in O<sub>2</sub> level to 2% increased the mean area of osteoclasts nearly 8-folds and exhibited a 13-fold increase in resorption lacunae in bone slices [151]. In another study, 2% O<sub>2</sub> exposure resulted in tenfold increase in resorption pit formation [149]. However, some studies also reported inhibition of osteoclast number and activity upon exposure to severe hypoxia which they attributed to extensive cell death following constant hypoxic exposure as reoxygenation after 2–3 days promoted osteoclastogenesis but a recent report also reports reduced osteoclast formation and function without affecting cell viability upon constant exposure of 1% O<sub>2</sub> [150, 152]. Although hypoxia has been reported to increase osteoclast number and activity, hypoxia was also shown to suppress OPG, preventing RANK-induced osteoclast formation and function [126, 142–144, 152–156]. Osteoclasts require high amount of ATP for their bone-resorbing function, and therefore, excessive accumulation of mitochondrial ROS during low oxygen tension for longer periods may promote apoptosis and limit bone resorption [157]. A significant decrease in bone calcium content with increased osteoclast differentiation after exposure to low oxygen levels has been reported in rainbow trouts which is consistent with the report of a fivefold increase in osteoclast-mediated calcium release after exposure to 2% O<sub>2</sub> in a 3-day organ culture of mouse calvarial bones [148, 158].

Knowles and Athanasou, for the first time, showed that HIF-1 $\alpha$  siRNA nullified the hypoxic increase in bone resorption [150]. HIF-1 $\alpha$  not only enhanced osteoclast-related RUNX2 and ALP gene as well as protein expression but also the number of TRAP-positive osteoclasts [159]. Shirakura et al. demonstrated that HIF-1 $\alpha$  knockdown increased OPG expression under hypoxic conditions [156]. Heterozygous deletion of PHD2 increased expression of proresorptive

**Table 3** Animal studies on the effect of hypobaric hypoxia on skeletal homeostasis in vivo

References	Sample			Outcome
	Sample size	Altitude	Duration	
Yin et al. [105]	SD rats (8 weeks) <i>n</i> = 20	4000 m	3 weeks	↓OCN ↓RUNX2 #BMD #BV #BV/TV #Tb.Th #Tb.N #ALP activity
Durand et al. [106]	Male C57BL/6 J mice (10 weeks) <i>n</i> = 40	19,000 m (50 kPa)	4 days	↓CFU-F pool ↓Ob.S/BS ↑Oc.S/BS #BV/TV
Wang et al. [98]	SD Rats (12 weeks) <i>n</i> = 8	6000 m	3 weeks	↓BV/TV ↓Tb.N ↓BMD-total ↑Tb. spacing #BS/TV #Tb. thickness ↓Maximum load ↓Structural stiffness ↓Maximum stress ↓Young's modulus
Lezon et al. [100]	Male Wistar rats (4 weeks) <i>n</i> = 20	5450 m	4 weeks	↓Femur length and weight ↓Cortical area ↓XCSMI ↓Load at fracture ↓Structural stiffness ↓Load at yielding #Young's modulus ↓Degree of mineralization
Bozzini et al. [99]	Female SD rats (30 days) <i>n</i> = 56	4100 m	2, 4, 6, 8 months	↓Load at fracture ↓Structural stiffness ↓Femur length and weight ↓Cortical area ↓CSMI →Elastic modulus
Bozzini et al. [101]	Female SD rats (32 days) <i>n</i> = 28	Hypoxia I 4100 m Hypoxia II 5450 m	42 days	↓Femur length and weight ↓Bone volume ↓Cross-sectional area ↓CSMI →Load at fracture →Structural stiffness →Load at yielding →Young's modulus →Elastic stress →Femur calcium mass #Calcium ash
Conti et al. [104]	Female Wistar rats (21 days) <i>n</i> = 30	16,000 m (506 mbar)	90 days	↓Femur length and weight ↓Femur ultimate load ↓Hemimandible (HM) length ↓HM weight ↓HM ultimate load



**Table 3** (continued)

References	Sample			Outcome
	Sample size	Altitude	Duration	
Bozzini et al. [102]	Female SD rats <i>n</i> = 20	5500 m	60 days	#Cross-sectional area ↑CSMI ↓Weight/length ratio ↑Horizontal diameter (outer and inner) #Vertical diameter (outer and inner) #Elastic limit #Ultimate load #Stiffness ↓Elastic modulus ↓Yield stress

↑, upregulated, ↓ downregulated, # unaltered, NR not reported, CFU–F colony–forming unit femur, OB.S/BS osteoblast surface area/bone surface area, OC.S/BS osteoclast surface area/bone surface area

genes in bone marrow cells, resulting in 3.7-fold higher resorption as compared to wild type [153]. Similarly, in vivo depletion of PHD2 led to increased bone resorption, reducing BV/TV and Tb.N, increased Tb.Sp suggesting the role of PHD2 and HIF-1 $\alpha$  in hypoxic-induced bone resorption [153]. Stimulation of proresorptive genes and glycolytic activity seems to be the mechanism by which HIF-1 $\alpha$  affects osteoclast function as knockdown of HIF-1 $\alpha$  suppressed hypoxia-mediated glycolysis and acid secretion for bone resorption [160–162]. It is well established that HIF-1 $\alpha$  induces an increase in osteoclast activity but its role in osteoclastogenesis needs more understanding. Digoxin, an inhibitor of HIF-1 $\alpha$ , has been reported to attenuate osteoclastogenesis through downregulation of RANK/RANKL signaling [163]. Several reports have similarly noted increased osteoclast differentiation following HIF-1 $\alpha$  stimulation [6, 164, 165], while a study noted that HIF1- $\alpha$  inactivation through siRNA accelerated osteoclast cell fusion and HIF-1 $\alpha$  induction moderately inhibited its differentiation [153]. In few reports, HIF-1 $\alpha$  induction caused a small decrease in osteoclast numbers, which authors reasoned to the longer reoxygenation time compared to other studies [148–151, 153]. Homozygous knockdown of PHD3 in animal models increased osteoclast formation, which was associated with increased expression of the differentiation marker NFATC1, which however, did not change the final number of osteoclasts formed but increased the final resorption activity [153, 166].

Initially, it was thought HIF-2 $\alpha$  had no role in osteoclast resorption as silencing of HIF-2 $\alpha$  in monocyte-derived osteoclasts had no effect on the hypoxia-induced resorption [150, 167, 168]. However, a recent study reported that HIF-2 $\alpha$  was capable of stimulating and accelerating osteoclastogenesis in M-CSF- and RANKL-treated osteoclasts [143]. The overexpression of HIF-2 $\alpha$  resulted in increased

osteoclast formation with large cytoplasmic compartments, suggesting its role in osteoclast maturation, similar to HIF-1 $\alpha$  [143, 153]. Similarly, inhibition of HIF-2 $\alpha$  led to reduced number of nuclei and expression of osteoclast-related genes finally reducing osteoclast differentiation. TRAF6 which is an adapter of the RANK gene may have role in HIF-2 $\alpha$  mediated osteoclastogenesis [143]. TRAF6 activates NFATC1 and promotes osteoclastogenesis [143, 169, 170]. HIF-2 $\alpha$  overexpression further enhanced TRAF6 expression in RANKL-mediated osteoclast differentiation. Inhibition of HIF-2 $\alpha$  lead to inhibition of TRAF6 and induced increase in osteoclast formation and differentiation [143]. Therefore, HIF-2 $\alpha$  may increase bone resorption by increased expression of osteoclast activity genes like TRAP and NFATC1 mediated through increased osteoclast number associated with HIF-2 $\alpha$  stabilization [143]. It is difficult to conclude the role of hypoxia on osteoclast as contradictory reports have emerged in various studies. Amount and duration of hypoxia may be playing role in determining whether hypoxia stimulates osteoclast growth and function or promote apoptosis through cell death. However major studies report hypoxia through HIF (1 $\alpha$  and 2 $\alpha$ ) to stimulate osteoclast number and function.

### Crosstalk Between Osteoclasts and Osteoblasts

Osteoclastogenic response to hypoxia may be mediated through both independent cell action as well as osteoblast–osteoclast crosstalk as osteoclast requires RANKL signals from osteoblasts for their proliferation, differentiation, and activity [166, 171–173]. Adenosine triphosphate (ATP) released from osteoblast in response to low oxygen tension was shown to inhibit bone formation while it stimulated bone resorption via the P2Y1 receptor and therefore could affect the balance between formation and resorption

of bone through local purinergic signaling [174, 175]. In presence of osteoblasts, the PHD inhibitor, FG-4592's ability to inhibit osteoclastogenesis was improved but bone resorption activity was reduced [166]. HIF-2 $\alpha$  was also reported to be an important mediator of osteoblast-osteoclast crosstalk as osteoclast-specific loss of HIF-2 $\alpha$  solely affected osteoclasts, whereas osteoblast-specific loss of HIF-2 $\alpha$  affected both osteoblasts and osteoclasts [143]. HIF-2 $\alpha$  seems to bind to RANKL promoter directly to increase osteoclast differentiation [143, 176] while the HIF target gene, OPG and its intermediary, interleukin 33, may be responsible for osteoblast-mediated inhibition of osteoclastogenesis [142, 144, 154, 155]. Though there are evidences of hypoxia-induced increase in osteoclast number to be the result of osteoblast-osteoclast crosstalk [126, 156, 173] but hypoxia in absence of osteoblast was also able to demonstrate increased differentiation of osteoclasts. Therefore, exploring downstream signaling might improve our understanding of this crosstalk [148, 149].

### Hypoxia and Osteocytes

Osteocytes contribute significantly to bone formation through  $\beta$ -catenin which is required for the osteoanabolic effect of mechanical loading and constitutively expressing  $\beta$ -catenin in osteocytes has been reported to increase bone mass in rat models [177, 178]. The disruption of the lacuno-canalicular network formed by osteocytes, necessary for nutrient and gaseous exchange, resulted in localized hypoxia in bone [179, 180]. Hypoxia caused by mechanical unloading has been reported to induce osteocytic apoptosis [181, 182].

One percent O<sub>2</sub> induced hypoxia after 16 h and up to 72 h. It significantly reduced osteocyte number at 8 and 48 h, induced cell death at 8, 24, and 48 h and induced apoptosis at 16, 24, and 48 h [183]. Acute oxygen deprivation at 2% O<sub>2</sub> for 4–12 h resulted in 2.1- to 3.7-fold upregulation of HIF-1 $\alpha$  protein expression in MLO-Y4 osteocyte-like cells compared to cells cultured in normoxia [184]. Osteocyte cells exposed to hypoxia simulated by 100  $\mu$ mol/L CoCl<sub>2</sub> or 2% O<sub>2</sub> stably expressed HIF-1 $\alpha$  proteins and upregulated the expression of RANKL at both gene and protein levels [185]. Also, knock-down of HIF-1 $\alpha$  in osteocyte cell lines downregulated the expression of RANKL [185]. Osteocyte-like cells exposed to hypoxia augmented secretion of chemotactic factors [186] and GDF15 to promote osteoclastogenesis [180] and influenced osteoblast-to-osteocyte transition [73]. Hypoxic osteocytes have been reported to increase their expression of OPN, a potent osteoclast chemotaxant, which increases the migration and attachment of osteoclasts [187].

Hypoxia with increased HIF-1 $\alpha$  expression was reported in mice osteocytes following mechanical unloading and disuse [184, 187, 188] and mice lacking

HIF-1 in osteocytes reported increased bone formation when subjected to tibia loading as a result of increased osteoblast activity [188]. Conditional knockout of PHD2 in osteocytes resulted in high bone mass phenotype and blunted osteoporotic bone loss induced by estrogen deficiency or mechanical unloading by enhancing bone modeling in young and adult mice while reducing bone remodeling in aged mice [189]. Mice conditionally lacking Von Hippel–Lindau (VHL) protein in osteocytes (10-kb *Dmp1-Cre*) exhibited a high-bone mass phenotype [190]. Osteocytic HIF-1 $\alpha$  not contributing to skeletal development has also been reported in one study where *Dmp1-Cre; Hif1af/f* mice were phenotypically indistinguishable from *Cre*-negative control mice, with insignificant differences in trabecular bone volume fraction, cortical area fraction, or bone formation rate suggesting that osteocytes utilize an alternate HIF- $\alpha$  isoform, or HIF-independent signaling, to influence the skeleton [190]. Reduced expression of HIF-1 $\alpha$  has also been reported to be involved in osteocyte-mediated osteoclastogenesis [191]. Osteocytes play a role in communication among cells in bone and reports indicate hypoxia to inhibit osteocyte growth and differentiations and promote osteoclast-mediated resorption but osteocytic HIF-1 $\alpha$  seems to increase bone mass through osteo-angiogenesis coupling in osteoblasts.

### Role of Wnt Pathway in Bone Mineral Metabolism

A variety of signaling pathways like Hedgehog (Hh), Wnt, BMP, and Notch pathway have been shown to function at specific and/or multiple stages in the differentiation cascade of osteoblasts and osteoclasts [20, 192, 193]. HA inhabitants and soldiers posted at these altitudes are exposed to a greater extent of external loads because of tough terrain and scanty transportation facilities [26]. Induction of Wnt signaling leads to load-induced bone formation in response to external loads [194–196]. Wnt family proteins are abundantly expressed in skeletal tissue and regulate endochondral ossification through both canonical and non-canonical pathways [197]. In the canonical Wnt pathway, Wnt proteins bind to Frizzled receptors and the Lrp5/6, which leads to the stabilization of beta-catenin ( $\beta$ -catenin) and its translocation to the nucleus to activate the transcription of target genes [198–200]. Regulation of Wnt signaling occurs via secreted decoy receptors or antagonists like sclerostin and DKK that bind to Lrp4-6 to prevent Wnt-Lrp interaction and signal transduction [196, 201]. Loss-of-function mutations in Lrp5 cause low bone mass condition: osteoporosis pseudoglioma (OPPG) and missense mutations lead to high bone mass phenotype [202, 203]. Activating mutations in Lrp4-6

promoted high bone mass phenotypes while complimentary phenotypes emerged from the deletion of *Lrp4/5/6* [177, 178, 188, 204–210]. Wnt/ $\beta$ -catenin signaling plays a crucial role in the osteogenic differentiation and maturation of osteoprogenitor cells by promoting RUNX2 expression [211, 212]. It has also been demonstrated that the activation of Wnt signaling pathway inhibits bone resorption by increasing the expression of OPG from osteoblasts and decreasing the secretion of RANKL, thus altering OPG and RANKL ratio opposing osteoclast formation [213]. In non-canonical pathway, G protein-coupled phosphatidylinositol and PKCD activation are involved in the Wnt-mediated osteoblast differentiation [214] while Wnt5a in the non-canonical pathway induces in vitro osteoblastogenesis and Wnt-5 $\alpha^{+/-}$  mice show bone loss presumably because of less osteoblast to adipocyte number ratio [215].

### Interaction of Wnt Pathway with HIF

Several in vivo studies in rats and mice have reported a decrease in bone strength parameters viz. maximum load, ultimate load, structural stiffness, load at fracture, load at yielding, and elastic modulus after exposure to hypobaric hypoxia [98–102, 104]. In vivo and in vitro evidences reveal functional crosstalks between Wnt and HIF signaling. HIF-1 $\alpha$  was reported to inhibit osteoblast proliferation by inhibiting Wnt pathway through downregulation of  $\beta$ -catenin, cyclin D1, and c-Myc [207]. Active  $\beta$ -catenin in osteocytes is essential for osteoanabolic effect of mechanical loading and increases both cortical and trabecular bone mass [216, 217]. Conditional knockout studies using *Bglap-Cre*, HIF-1 $\alpha$ KO mice displayed increased load-induced bone formation through  $\beta$ -catenin and *Bglap-Cre*; VHLcKO mice showed attenuated osteocyte differentiation where both Osterix, the osteoblast-specific transcription factor, and HIF-1 $\alpha$  cooperatively reduced Wnt signaling [79, 218]. Contradictory reports have also indicated that hypoxia or hypoxia mimetics increased Wnt signaling in bone [219, 220]. Two recent reports demonstrated a relationship between hypoxia and Wnt/ $\beta$ -catenin signaling in osteocytes: VHL and PHD2 deletion in osteocytes decreased sclerostin and increased canonical Wnt signaling leading to increased bone mass [36, 221]. Activation of HIF signaling in osteocytes epigenetically repressed sclerostin expression resulting in high bone mass phenotype caused by increased bone formation and decreased bone resorption [21, 207, 210]. Hypoxia was reported to decrease sclerostin levels and increase Wnt signaling while a contradictory report of increased sclerostin levels by 62% when compared to the normoxia group has also been reported in cell culture studies [208, 222].

### Role of Estrogen in Bone Mineral Metabolism

Reports have associated exposure to HA with disturbance in steroidogenesis which was more prominent during early exposure [223]. Higher levels of augmented nitric oxide (eNOS) which activated estrogen in both young and middle-aged Ladakhi women was reported and similarly increased levels of circulating estrone and 17 $\beta$ -estradiol as a result of greater aromatase activity were reported during HA residency [224–226]. The same study also reported increased mRNA levels of estrogen receptors at HA [226]. It is well established that estrogen protected women from bone loss and post-menopausal women undergo more bone resorption than formation leading to bone loss in old age [227]. An interventional study had reported that estrogen accounted for more than 70% of the effect of sex steroid hormone on bone resorption in men and studies since then have reported that bioavailable estrogen which maintains cortical bone constituting nearly 80% of bone mass, is more closely associated with bone density than bioavailable testosterone in both male and female [52, 228–232]. Estrogen has been reported to decrease the number, activity, and apoptosis of osteoclasts leading to decreased bone resorption while increase osteoblast proliferation, differentiation, and activity which is therefore reduced estrogen levels at old age leads to loss of bone formation [233–236]. Though contradictory reports have also been reported, estrogen has also been reported to disrupt absorption of parathyroid hormone (PTH), which extracts calcium from bone to maintain systemic calcium levels and therefore promotes bone formation [237]. In the animal model, ovariectomy lead to decreased bone strength, distorted bone microarchitecture, and increased markers of bone turnover, serum ALP, serum OCN, urinary calcium, urinary phosphorus, and urinary Cr [238]. Authors of the same study noted increased expression of HIF-1 $\alpha$  and NF- $\kappa$ B and decreased expression of VEGF and  $\beta$ -catenin after ovariectomy [238].

### Interaction of Estrogen with Wnt Pathway and HIF Pathway

Estrogen has been reported to increase osteoblast-mediated production of transforming growth factor- $\beta$  (TGF- $\beta$ ), resulting in increased Wnt production by osteoclast [239]. Mice with *Lrp5* mutations were resistant to sclerostin and had no or reduced bone loss following ovariectomy [240]. Human studies have provided considerable evidences of estrogen regulating the production of sclerostin [241]. Treatment of postmenopausal women with estrogen, or raloxifene, an estrogen receptor modulator, reduced circulating sclerostin levels [242–245], and correspondingly, bone sclerostin mRNA levels were also lower in the estrogen-treated women [242].

These studies are consistent with the observation that mice with osteocyte-specific deletion of estrogen receptor  $\alpha$  had increased bone sclerostin mRNA levels [246]. Inhibition of sclerostin using a humanized monoclonal antibody, romosozumab, resulted in an increase in bone formation and reduction in bone resorption, qualitatively similar to results of estrogen treatment in postmenopausal women [241, 247]. These studies indicate a potential role for sclerostin, the inhibitor of the Wnt pathway in mediating estrogen-deficiency-mediated bone loss but the underlying mechanism of this effect needs further investigations.

Studies have also revealed crosstalk between estrogen signaling pathway and HIF- $\alpha$  regulation in bone remodeling [6, 248, 249]. 17 $\beta$ -estradiol was reported to increase HIF-1 $\alpha$  and VEGF protein levels and partially stimulate MSC proliferation and HIF-1 $\alpha$  was shown to be required for osteoclast activation in postmenopausal osteoporosis [6, 250]. Bone density, bone vessels, and bone formation were reported to be reduced in HIF-1 $\alpha$  conditional knockout (KO) ovariectomized mice when compared to wild-type ovariectomized mice [248]. The expression of HIF-1 $\alpha$  and VEGF decreased in ovariectomized mice but not in VHL KO ovariectomized mice while activation of the HIF- $\alpha$  pathway increased angiogenesis as well as osteogenesis thereby conferring protection from ovariectomy-induced bone loss [249]. These reports are consistent with the study by Yen et al., who demonstrated that diosgenin, having estrogenic effects, induced HIF-1 activation and angiogenesis through the SRC kinase, P38 MAPK, and AKT signaling pathways in osteoblasts [251]. These reports therefore point towards the role of decreased stimulation of HIF-1 $\alpha$  and angiogenesis contributing to bone loss during estrogen deficiency. Though far stretched, reports by various authors give an impression of crosstalk among HIF signaling, Wnt pathway, and estrogen signaling which may be regulating bone mineral homeostasis in HA environments.

## Summary and Conclusion

This review aims to provide available data on the role of hypoxia in bone health in relation to the Wnt pathway and estrogen at HA. Studies indicate a proportional relationship between altitude and bone health which deteriorates with increasing altitude. BMD and bone strength parameters have been reported to be decreased by various groups in both human and animal models respectively. Biochemical and endocrinal indicators of bone health also indicate decreased bone turnover at HA as compared to sea level. Duration of stay or exposure is also an important parameter along with altitude and therefore oxygen concentration, while assessing the role of hypobaric hypoxia on bone health. Extended stay as well as higher altitudes

proportionally increased the deterioration of bone quality in human and animal studies. Crosstalks among the mediators of the hypoxia-sensing pathway, Wnt pathway, and estrogen have been reported in various studies which need validation in relation to bone health and mineral metabolism at HA. In conclusion, it is evident that there is only a handful of reports on the effects of hypobaric hypoxia on bone health. The tough terrain is a major challenge to conduct studies at HA. Also, there are many factors such as cold, dehydration, and high UV radiation operating simultaneously at these altitudes making it difficult to isolate the effect of hypobaric hypoxia on bone health from human studies. Major instruments used for measuring BMD, define osteopenia and osteoporosis based mainly on *T* score values prepared from a reference database of the western population which may not be reflecting the true bone health status of the Indian population due to geographical and ethnic differences. Therefore, detailed studies are required to prepare a database for the Indian population.

**Data Availability** Not applicable.

**Code Availability** Not applicable.

## Declarations

**Ethics Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent for Publication** Not applicable.

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