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Research progress on the role of cold-sensitive channel TRPM8 in controlling low temperature-induced bone metabolic imbalance

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Abstract

With increasing aging population, osteoporosis has emerged as a public health problem worldwide. Epidemiological data reveal that the prevalence of osteoporosis in cold regions is high, and low temperatures may crucially affect bone mass. Recent studies have found that the transient receptor potential melastatin-8 (TRPM8) channel, a cold-sensitive ion channel, can sense cold environment, and can be activated in cold environment. It may play an antagonistic role in low temperature-induced bone mass reduction. Mechanistically, this function may be ascribed to the activation of TRPM8 channel proteins in human bone marrow mesenchymal stem cells (hBM-MSCs), which causes osteoblast differentiation and mineralization in the bone. TRPM8 channel on the surface of brown adipocytes participates in the thermogenesis in brown adipose tissue (BAT) and the regulation of whole-body energy balance to maintain bone homeostasis. TRPM8 may be involved in bone remodeling throughout life. This paper reviews recent research on the possible antagonistic mechanism of TRPM8 in signaling pathways related to low temperature-induced bone mass loss and assesses the possibility of TRPM8 as a molecular target for the prevention and treatment of low temperature-induced osteoporosis in cold regions.

Keywords

TRPM8; low temperature; osteoporosis; bone mass

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Osteoporosis is a systemic metabolic bone disease characterized by a decrease in bone mass and deterioration of bone microstructure, leading to increased bone fragility and the occurrence of fracture. Previous studies have shown that bone tissue is composed of bone remodeling units, formed by osteoclast-osteogenesis coupling. The process starts with osteoclasts absorbing old bone and developing new bone. Under physiological conditions, bone resorption and formation are in balance. However, in patients with osteoporosis, a combination of factors, including genetic, environmental, diet, and lifestyle, eventually lead to an imbalance in bone remodeling[1-2].

Several epidemiological studies have shown that exposure to low ambient temperature increases the risk of overall mortality from many diseases, including osteoporotic fractures, especially during extreme weather events such as cold spells[3-6]. Results from a nationwide, longitudinal cohort study from Norway showed a progressive increase in the incidence of and mortality due to osteoporotic fractures of the forearm and hip with decreasing temperature, with a stronger association among older adults and gender differences in fracture types[7]. Several studies from Japan, the United Kingdom, Spain, Canada, and Australia have also shown an increase in hip fracture risk with decreasing temperature[8-11]. Cold temperature may be an "underestimated risk factor" for osteoporosis-related diseases. Therefore, fully understanding the pathogenesis of special bone loss in cold regions and formulating prevention and treatment strategies to reduce the prevalence of osteoporosis and fracture in older adults in cold regions are of paramount significance. In addition to the uncontrollable factors, recent studies have found that low temperature may be one of the main determinants affecting the regulation of bone metabolism and decreasing bone mass.

Existing epidemiological data show that low levels of vitamin D and parathyroid hormone caused by long-term low-temperature exposure and low sunshine increase bone loss. Loss of

coordination and reaction time due to slippery roads, poor visibility, and excessive clothing all increase the risk of falls, leading to a significant increase in the incidence of osteoporosis and fracture[12-13]. Low temperature per se can directly reduce bone mass, and TRPM8 channel, which is the most important mediator of cold sensation, involved in antagonizing the negative effect of low temperature on bone mass. This paper reviews recent research on the important role of TRPM8 as a mediator of cold sensation *in vitro* and the possible underlying mechanism for bone protection. We also discuss the possibility of using TRPM8 as a target for the prevention and treatment of osteoporosis caused by low temperatures in cold regions.

1 Low temperature decreases bone mass

The adverse effects of low temperature on bone mass acquisition have been verified in many animal studies. In rodents, bones respond dynamically to environmental temperature. Skeletal phenotypes in different housing temperatures may not be entirely determined by genetic program. Animal studies have found that rearing mice at cold or room temperature results in limb bones of varying lengths[14]. Compared to the room-temperature group, mice in the low-temperature group eat more but have less body fat. The volume fraction, thickness, and junction density of bone trabeculae in the distal femur and the area fraction of bone in the middle femur are significantly lower in the low-temperature group than those in the room-temperature group. Moreover, the expression of uncoupling protein-1 (UCP-1) in brown adipose tissue (BAT) correlates negatively with temperature. The results support the hypothesis that low temperature is adverse to bone mass acquisition, and up regulation of UCP-1 in BAT leads to increased non-shivering thermogenesis, which may reduce bone loss but is insufficient to prevent the eventual loss of bone mass[15].

The traditional explanation for temperature-induced growth effects on skeletal extremities is changes of blood supply of essential nutrients and growth factors due to temperature-induced vasoconstriction or vasodilation. The detrimental effect of blood flow disruption on bone growth is well-known. Ambient temperature may affect limb size by regulating peripheral tissue temperature to modulate cell proliferation and matrix production in cartilage. Thus, vasoconstriction and vasodilation may affect limb growth by regulating the temperature within the developing cartilage instead of nutrients and hormones supplies[16-17].

Low temperature can affect bone tissue metabolism through multiple pathways to damage bone structure, leading to osteoporosis. However, the regulatory pathways key to this process are not fully understood. Studies have shown that the transient receptor potential (TRP) melastatin-8 (TRPM8), a cold-sensitive ion channel, participates in bone metabolism through various mechanisms upon receiving cold signals to antagonize the effect of low temperature on bone mass reduction.

2 TRPM8: a cold-sensitive ion channel

Human psychophysical research suggests the presence of a clear and reproducible dividing line between harmless and damaging temperature perception, which allows us to identify and avoid temperature stimuli that cause bodily damage. This autoprotective mechanism relies on several TRP channels that sense temperatures of different ranges[18]. TRP channels are widely distributed in various mammalian tissues and cells and include canonical (TRPC1−7), vanilloid (TRPV1−6), melastatin (TRPM1−8), ankyrin (TRPA1), mucolipins (TRPML1−3), and polycystins (TRPP3 and TRPP2), comprising six subfamilies. As gated molecules in the receptor system, TRP channels are composed of six transmembrane (TM) domains and intracellular N- and C-terminals. TM5 and TM6 near the C-terminal form the ion micropore region, the key site that determines the temperature, voltage, and ligand sensitivity of TRP channels. TRP channels are non-selective cationic channels with high permeability to calcium ions (Ca^{2+}), and they act as "cellular sensors" in response to changes in the cellular environment, including temperature, stretching/pressure, chemicals, oxidation/reduction, osmotic pressure, and pH, and convert stimuli from the external environment to inward currents[19-20]. Different TRP channels sense different ranges of temperature. These temperature-sensitive TRP channels are collectively called thermoTRP channels (thermoTRPs)[21-22]. ThermoTRPs can sense temperatures across the entire physiological spectrum, from painful searing heat to comfortable warmth and cool, and biting cold. ThermoTRPs can also be activated by chemical ligands including menthol, capsaicin, allicin, cannabinoids, and cinnamaldehyde. Eleven thermoTRPs have been identified in mammals, including the heat receptors, TRPV1-TRPV4 and TRPM2-TRPM5, and cold receptors, TRPM8 and TRPA1[23-27].

The human gene encoding TRPM8 is located on chromosome 2 (2q37.1) and is approximately 102.12 kb in length. It is composed of 24 exons. TRPM8 is a gene highly homologous to the family of TRP channels identified by Tsavaler *et al.* using the cDNA library. It was later named TRPM8[28]. TRPM8 channel proteins are widely distributed in the peripheral dorsal root ganglion and central trigeminal neurons. TRPM8 channel is distributed in vascular smooth muscles, lung, skeletal muscles, human bone marrow mesenchymal stem cells (hBM-MSCs), BAT, mesentery, gastric fundus, liver, bladder, prostate, breast, thymus, skin, and other tissues and organs[29-30]. TRPM8 channel, known as the "cold channel"[31-33], can be activated to conduct Ca^{2+} influx in cold environments (8°C-28°C) and by menthol,

nicolin, and icilin (synthetic cooling compounds).

Under physiological conditions, TRPM8 channel acts as a cold receptor in the somatosensory system to conduct non-invasive temperature stimuli and initiate cold perception. Studies have shown that TRPM8-knockout (TRPM8-KO) mice lose their menthol and cold-induced responses in neurons and nerve fibers, thereby failing to seek comfortable warm zones in cold environments. Normal animals, however, remain resistant to harmful cold stimuli. The TRPA1 channel responds to low temperatures (<18°C), cannabinoids, mustard oil, and other isothiocyanates, as well as mechanical stimulation (inner ear hair cells), but not to menthol. TRPA1-knockout mice are insensitive to allicin and mustard oil, but retain their cold-evoked response with intact behavioral responses to cold stimuli. These findings suggest that TRPM8 channel, but not TRPA1 channel, plays an important and dominant role in mediating cold sensation. Evidence also suggests The fact that some cold-responsive neurons in the posterior root and superior cervical ganglia do not respond to menthol or mustard oil also suggest there may be some other cold-sensitive receptors in addition to TRPM8 and TRPA1 channels[34-36].

The discovery of TRPM8 reveals how the body senses temperature. Early research in the field focused on temperature and pain perception. Certain members of the TRP family have been used as interventions for the treatment of clinical pain and hypothermic injuries[25,31]. However, recent studies suggest that dysfunction of TRPM8 is associated with many diseases, and its roles in the cardiovascular and metabolic fields, cancer, endocrine, urinary, respiratory, and digestive systems have attracted widespread attention. Research on the therapeutic potential in this context has expanded from cancer to neuropathic pain, dry eye disease, obesity, hypertension, irritable bowel syndrome, chronic cough, and other diseases[37-38]. Temperature sensing by TRPM8 channel and the sensory neuron system have remained the focuses of research. With research progress in the mechanisms underlying temperature sensing at the molecular level, our understanding of body temperature regulation, heat limit mechanism, and other sensory functions is expected to deepen.

3 Low temperature activates TRPM8 in hBM-MSCs promoting osteoblast differentiation

HBM-MSCs express TRPM8, a critical Ca^{2+} channel associated with bone mineralization. The subcellular distribution pattern of TRPM8 channels in hBM-MSCs was measured in permeable cells by confocal immunofluorescence microscopy, and the results showed that they were diffusely distributed in hBM-MSCs[39]. This was followed by patch-clamp recordings of whole-cell current in the presence of TRPM8 channel agonists or antagonists. The results showed that menthol significantly enhanced the outward current at depolarizing potentials, which was blocked by 26% after exposure to N-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carbox-amide (BCTC), a TRPM8 channel antagonist. The results of this study showed that hBM-MSCs express menthol-activated BCTC-sensitive currents with strong outward rectifying properties, resembling the currents carried by TRPM8 channels, as reported previously in other natural cells. This indicates that hBM-MSCs abundantly express TRPM8 channels[39].

It is well known that calcium in the body is closely related to bone balance. On the one hand, bones depend on an adequate supply of calcium to maintain their structural and mechanical properties; on the other hand, bone is a calcium reservoir from which calcium can be mobilized to maintain its normal levels in the blood. Calcium imbalance compromises the bone integrity. Bone homeostasis also depends on the normal differentiation and function of bone cells, which are largely determined by calcium signaling within cells. Many studies have identified intracellular Ca^{2+} elevation due to its influx or endoplasmic reticulum depletion as a common mechanism for activating different transcription factors involved in osteogenic differentiation in BM-MSCs obtained from different sources[40-42]. TRPM8 is a voltage-gated Ca^{2+}-dependent Ca^{2+} channelin excitatory cells (*e.g.*, neurons and muscles). It is also a non-voltage-gated Ca^{2+}-dependent Ca^{2+}channel in non-excitatory cells (*e.g.*, epithelial, endothelial, mucosal, and tumor cells). Changes in intracellular Ca^{2+} concentration play an important role in cellular functions, like muscle contraction, the release of neurotransmitters or modulators, cell differentiation and proliferation, gene transcription, and cell death[43-45]. In addition to its expression on the surface of hBM-MSCs, TRPM8 is also expressed in the endoplasmic reticulum, suggesting that its main function may be related to intracellular Ca^{2+} release. Thus, TRPM8 plays an important role in both extracellular Ca^{2+} influx and mobilization of intracellular Ca^{2+} stores into the cytosol[39].

One study assessed the osteogenic differentiation of hBM-MSCs by adding osteogenic differentiation mediators in the presence or absence of TRPM8 channel agonists menthol or nicolin, or TRPM8 channel antagonists, BCTC, and stained Ca^{2+} deposits in differentiated cells with alizarin red dye. Menthol and nicolin, promoted the differentiation of osteoblasts and intracellular Ca^{2+} deposition. Osteogenic differentiation decreased by BCTC[39]. These results suggest that the activation of TRPM8 favors osteogenic differentiation, which is dependent on Ca^{2+} influx and Ca^{2+} mobilization from endoplasmic reticulum, thereby controlling skeletal homeostasis. Future studies may aim to decipher the intrinsic mechanisms by which TRPM8 activation in hBM-MSCs promotes osteogenic differentiation and the associated signaling pathways.

TRPM8 channel, which plays a key role in mediating cold sensation, can promote osteogenic differentiation by regulating Ca^{2+} distribution. Low temperature-mediated TRPM8 activation in hBM-MSCs may contribute to bone reconstruction and remodeling throughout the life cycle. In men, TRPM8 is necessary for the regulation of normal body temperature and maintenance of bone density, whereas in women, TRPM8 primarily controls the fat content in the bone marrow. Bone marrow adipocytes and osteoblasts share common BMSCs, both of which are known to increase significantly at low bone density. In a study on the role of TRPM8 in regulating bone density and microstructure, the researchers placed female and male wild-type (WT) mice and TRPM8-KO mice in a freezer (4˚C) and examined the bone marrow adipose tissue (BMAT) and bone microstructure transformation[46]. Their results showed that the bone density in male TRPM8-KO mice was significantly lower than that in WT counterparts, and similarly, the bone femur length and cross-sectional area were smaller with TRPM8-KO than with WT, suggesting impaired cold adaptation after TROM8 deficiency. Compared to WT mice, BMAT expression was increased, and the microstructure parameters of femoral cortical bone and vertebral spongy bone were reduced in female TRPM8-KO mice. However, TRPM8 deficiency was not required for cold-induced trabecular loss in both sexes but the BMAT in female was significantly inhibited after TRPM8 knockout. The study also identified gender differences in the roles of TRPM8 in maintaining bone microstructure and BMAT[46]. An epidemiological study reported a strong association between temperature and the risk of forearm fractures in Norwegian, which was age- and gender-dependent being stronger with increasing age and in women than in men (61% *vs.* 27%, higher incidence)[7]. Similar female vulnerability to osteoporotic fractures was also reported in other countries[47-50]. These epidemiological observations are consistent with the results of gender differences in the role of TRPM8 channels in maintaining bone microstructure.

In conclusion, low temperature up-regulates some key osteogenic factors by activating TRPM8 in hBM-MSCs to elevate intracellular Ca^{2+} concentration and promote osteoblast differentiation and mineralization, thereby minimizing low temperature-induced bone mass reduction. However, identifying the specific and precise signaling mediators involved in this pathway awaits further in-depth studies.

4 TRPM8 promotes BAT thermogenesis and maintains energy balance in the body to regulate bone homeostasis

TRPM8 channels are also expressed on the surface of BAT cells. Mice exposed to cold or treated with menthol show increased adipocyte differentiation and energy expenditure through activating TRPM8 channels expressed in brown adipocytes. Moreover, the expression of UCP-1 gene in mitochondrial intima is enhanced, leading to enhanced oxidative respiration chain uncoupling and heat generation, thereby increasing the core body temperature. Activation of TRPM8 channels can also enhance the "browning" of mouse white adipose tissue, heat production, and sugar utilization in BAT, improve sugar metabolism and adipose tissue hyperplasia, inhibit fat and insulin resistance, and prevent obesity induced by high-fat diet in mice. Moreover, TRPA1- and TRPM8-expressing sensory nerves also contribute to the potentiation of BAT thermogenesis and energy expenditure in mice[51-52]. During cold exposure, TRPM8-KO mice and WT litter mates treated with TRPM8 channel antagonists show decreased core body temperature and are prone to obesity. TRPM8 has been studied as a potential target for obesity treatment. TRPM8 is expressed in C2C12 muscle cell lines, and its activation increases energy expenditure and improves skeletal muscle endurance during exercise in mice[53].

The positive relationship between BAT and bone mass has been confirmed, and BAT activation positively affects bone structure. BAT function can directly or indirectly affect bone metabolism. As an independent predictor of bone mass, BAT has a positive predictive power on femur structure[54-57]. When adapting to low temperatures, the sympathetic nervous system activates BAT to increase fat oxidation and energy consumption. This process is mediated by increased UCP-1 expression, leading to increased heat production to maintain body temperature. Studies have shown that minor cold exposure had significant effects on energy expenditure and UCP-1 levels with 43% and 400%increases, respectively, in mice reared at medium temperature (29°C) and room temperature (22°C). The thermogenic activity of human BAT correlates negatively with energy metabolism disorders caused by aging, diabetes, and obesity. Moreover, the above three conditions are related to a decrease in bone mass and increases in fat volume in the bone marrow cavity and the incidence of fractures[58-60]. High BAT activity in healthy young women correlates positively with high bone density, and BAT volume, bone mass, and femur cross-section size in children and adolescents are also positively correlated. Women with cold-induced BAT have higher bone mass than those with a history of wasting diseases like anorexia nervosa, wherein the BAT function is lost[61-62].

Data from animal models suggest that BAT may play a positive role in bone mass changes after cold exposure. To clarify the function of BAT, some research teams have explored the influence of BAT on bone metabolism during low temperature-induced bone mass loss and the underlying mechanisms. In one such study, mice were exposed to low temperatures (4°C). The volume, bone remodeling, and microstructure of BAT, as well as the serum levels of C-terminal

telopeptide of type I collagen, CTX-1 (CTX-1), procollagen I N-Terminal propeptide (P1NP), and interleukin (IL) -6, were measured at 1, 14, and 28 days after cold exposure, respectively, and the effects of BAT on osteoclasts were investigated. Bone volume fraction was found to be significantly decreased, BAT volume increased, and IL-6 levels increased after 14 days of cold exposure. Dual labeling and *in vivo* TRAP staining showed changes in bone remodeling after cold exposure. BAT medium promoted BMSC mineralization but inhibited bone resorption by increasing the expression of osteocalcin, RUNX family transcription factor 2, and alkaline phosphatase. The results of this study suggest that the recovery of bone volume after cold exposure may be related to increased BAT activity. Thus, BAT may have a direct beneficial effect on bone mass by promoting osteogenesis and inhibiting osteoclast generation[60,63]. In conclusion, after receiving the cold stimulation, TRPM8 channels on the surface of brown adipocytes are activated by the sympathetic nerve, which in turn enhances the expression of UCP-1 in mitochondrial intima, promotes osteogenesis, and inhibits osteoclasts to produce direct protective effects on the bone mass.

Moreover, it is well-known that bone metabolism is closely related to the regulation of energy balance in the whole body[64-66]. Temperature changes have a profound effect on energy metabolism, which in turn affects bone remodeling. TRPM8 is important for maintaining body temperature in male mice, and its deficiency or blockade can trigger a compensatory response to bone mass reduction to offset the effect of a decrease in the core temperature[46]. Recent studies have shown increased bone formation and lower levels of bone resorption at the distal femur in mice raised at thermoneutral temperatures (32°C) compared to those raised at room temperature (22°C), which may be related to the weakening of this compensatory response[67-68]. The above findings suggest that the sympathetic nervous system-mediated stress response that transfers energy from bones to maintain body temperature balance in cold environments may be an important factor leading to bone loss. TRPM8 can antagonize this compensatory response through promoting hyperthermia by promoting UCP-1 over expression in BAT, but this is insufficient to prevent bone loss.

A possible coordinating mechanism for competing UCP-1 activity and neuropeptide Y (NPY) levels has recently been identified in mice after cold exposure. Hypothalamic NPY levels decreased after exposure to low temperature in UCP-1 deficient mice. Increased UCP-1 expression levels may stimulate increased hypothalamic NPY release, which subsequently inhibits sympathetic nerve activity and UCP-1 levels, thereby reducing BAT activity and limiting energy utilization. This is the case where the central NPY is a limiting factor to reduce energy loss as heat maintains the overall energy balance to maximize survival under environmental pressures[69-70]. When comparing the mice raised at medium temperature (29°C) and at room temperature (22°C), respectively, the bone mass, the mineral content and bone volume of the femur in the latter group were all significantly lower than the high-temperature group. Importantly, NPY-deficient mice did not show these cold-induced bone changes, whereas energy expenditure was significantly greater, suggesting that inhibition in non-thermogenic tissues, like bone, contributes to the adaptive response to cold exposure. This work confirms that NPY plays a key role in coordinating energy and skeletal homeostasis by inhibiting energy expenditure, UCP-1 levels, and bone mass under cold conditions[71]. This study also verified the beneficial effect of body temperature maintenance on bone mass after cold exposure through the activation of TRPM8 channels to promote heat production in BAT.

5 Conclusion

The above studies suggest that prolonged exposure to low temperatures may damage the bone structure. The activation of TRPM8 channels expressed on the cytoplasmic membrane of hBM-MSCs favors osteoblast differentiation and mineralization in osteoblasts, whereas inhibition of TRPM8 channels mitigates the beneficial effects on the bone mass reduction caused by low temperature. The same mechanisms may also apply to TRPM8 channels expressed on the surface of brown adipocytes, which contributes to BAT thermogenesis and energy balance, thereby maintaining bone homeostasis, in the body. Therefore, TRPM8 channel receptors might be considered a promising therapeutic target. The search for effective TRPM8 channel modulators, including agonists and antagonists, as well as TRPM8 diagnostic markers has attracted extensive attention.

Although many different TRPM8 modulators have been developed, most of them are still in the preclinical stage. Given the widespread distribution of TRPM8 channel proteins and their complex pathophysiological functions, the selectivity of TRPM8 modulators is a key, but largely unresolved, issue. Meanwhile, due to the sequence similarity or conservation and cross-talk among different subtypes of TRP channels, comprehensive delineation of the structural and functional differences between TRPM8 and other ion channels should provide a guideline for the validation of TRPM8 as a potential therapeutic target for the treatment of TRPM8-associated diseases and help generate new strategies and measures for the prevention and treatment of osteoporosis in cold areas.

Conflicts of interests

The authors declare no conflicts of interests.

Author contributions

Zhou P conceived the present idea and was in charge of overall direction. Zhang Y M carried out the literature search and wrote the first draft of the manuscript. Kazakova E. V and Chai H J completed the literature screening and manuscript revision. All authors provided critical feedback and helped shape the analysis and manuscript.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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