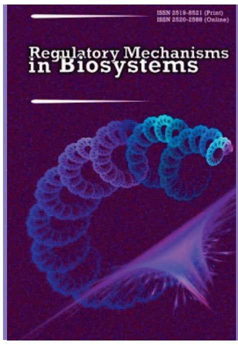

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Temperature, heat shock proteins and growth regulation of the bone tissue

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Ambient heat modulates the elongation of bones in mammals, and the mechanism of such a plasticity has not been studied completely. The influence of heat on growth and development of bone depends on its values. Five zones of temperature influence on the bone tissue with different biological effects have been distinguished : a) under-threshold thermal zone < 36.6 ºC, insufficient amount of heat is a limiting factor for osteogenesis; b) normal temperature zone 36.6–37.5 ºC, the processes of breakdown and development of bone in this temperature range is balanced; b) zone of mild thermal shock 39–41 ºC, the processes of functioning of osteoblasts, osteocytes and formation of the bone tissue intensify; d) the zone of sublethal thermal shock > 42 ºC, growth of bone slows; e) zone of non-critical shock > 50 ºC, bone tissue cells die. We propose a model of the mechanism of influence of heat shock on bone growth. Mild heat shock is a type of stress to which membrane enzymes adenylyl cyclase and cAMP-protein kinase react. Protein kinase A phosphorylates the gene factors of thermal shock proteins, stress proteins and enzymes of energy-generating processes – glycolysis and lipolysis. Heat shock protein HSP70 activates alkaline phosphatase and promotes the process of mineralization of the bone tissue. In the cells, there is intensification in syntheses of insulin-like growth factor-I, factors of mitogenic action, signals of intensification of blood circulation (NO) and synthesis of somatotropin. The affinity between insulin-like growth factor I and its acid-labile subunit decreases, leading to increased free and active insulin-like growth factor I. Against the background of acceleration of the capillarization process, energy generation and the level of stimulators of growth of bone tissue, mitotic and functional activities of producer cells of the bone – osteoblasts and osteocytes – activate. The generally known Allen's rule has been developed and expanded: "Warm-blooded animals of different species have longer distal body parts (tails) if after birth the young have developed in the conditions of higher temperature". The indicated tendency is realized through increased biosynthesis of heat shock proteins and other stimulators of growth processes in the bone tissue.

Keywords: thermal zones; osteogenesis mechanism; adenylyl cyclase; protein kinase; Allen's rule.

Introduction

The search for literature in our review was based on the abovementioned key words in the electronic data base PubMed. The search terms for the mechanism of the temperature influence on growth of the bone tissue were typed in different combinations using the list of the literature in original articles and reviews.

The ambient temperature and physical activity model the elongation of limbs of mammals, and the mechanisms this plasticity is based on is the mystery of the century. Heat and prolonged physical loads promote bone elongation (Ota et al., 2017). These factors contribute to increase in the delivery of soluble substances needed for growth of the epiphysial plate (Robbins et al., 2018; Chevalier et al., 2020; Starling, 2020).

The relevance of using thermal effect to promote bone growth is closely related to the practice of surgical correction of paired limbs (arms and legs) with disproportions in size and weight in people (Zhang et al., 2010; Racine et al., 2018). In cases of asynchrony of legs and arms, a longer limb is shortened by constant growth arrest or the shorter limb is lengthened when the difference is greater than 5 cm (Stevens, 2016). At the same time, the growth can be corrected painlessly by temperature through changes in the expression of insulin-like growth factor. Modern protocols of bone elongation include invasive operations or treatment schemes which are only partly efficient, while thermal influence leads to increase in the limbs' length. Heating can non-invasively increase the elongation of bones of different lengths in people (Serrat et al., 2015).

Thermotherapy is used to combat tumours. Osteoid osteomas are benign tumours that affect bones of up to 1.5 cm in diameter. Among the standard methods of their treatment, ray therapy and chemotherapy are used most often, combined with surgery mostly for patients with bone fractures. These treatments have a number of disadvantages and limitations. Therefore, over the recent decades, the means of hypothermal effect have been successfully implemented,which are characterized by an immediate pain mitigation (Moynagh et al., 2018; Tomasian & Jennings, 2020; Cazzato et al., 2021) and are recommended by the National Comprehensive Cancer Network (Heymann, 2021). Visiting a Fninish sauna improves the parameters of the muscular and bone masses (Toro et al., 2021). We should also note the distinguished stages of the mechanism – remodeling and developing the bone tissue (Povoroznyuk et al., 2021), the parallels in the mechanism of growth stimulation of the bone tissue under the influence of temperature and plyometry (Kuibida et al., 2021), the role of heat shock proteins (Leicht et al., 2019) and insulin-like growth factor-1 in the process of osteogenesis (Racine & Serrat., 2020).

Therefore, determining the mechanism how the temperature influences osteogenesis is a relevant theoretic and practical issue. The goal of the study was to reach a deep understanding of one of the aspects of regulating the growth of the bone tissue. Knowledge of the mechanism of influence of temperature on the elements of the skeleton is needed for comprehensive use of thermotherapy in medicine and sports, health-supporting technologies and understanding of patterns of the influence of abiotic ecological factors on morphogenesis of animals.

Influence of temperature on bone growth

Temperature models the energy metabolism that influences the process of remodeling of bones. At the same time, the mechanism through which the heat influences osteogenesis remains currently unknown in de-tail. In one experiment, two month-old male mice C57BL/6J were exposed to cold (4 °C) and normal (23 °C) temperatures for 28 days. Cold increased the apoptosis of osteocytes and decreased the length of the canals. Those changes were accompanied by decrease in the number of osteocytes that were positive to E11 (transmembrane glycoprotein, important for differentiation of osteocytes, first of all prolonging dendrites) and MMP13 (matrix metallopeptidase that breaks down the collagen I in extracellular matrix and potentially plays a role in rotating the articular cartilage) after 14 days. The indicated parameters returned to the initial levels after 28 days. The study revealed that after 14 days of influence of low temperatures the volumetric bone fraction significantly decreased, but recovered after 28 days. Brown adipose tissue affected remodeling of the bone by increasing thermogenesis (Du et al., 2021).

Heat (34 °C) protects the bone from loss of the bone tissue following surgery and removal of the ovaries. Thermotherapy increased the volume of trabecular bone, density of the connection and thickness, leading to improvement of biomechanical density of bones in adult females and young male mice. Those data indicate that heat influence is a potential variant of treating osteoporosis of bones, and thermotherapy may provide a mechanical basis for treating bone diseases (Chevalier et al., 2020). During osteoporosis, the bone loses weight, which heightens the risk of bone fracture. Heat prevented the fractures of femurs and loss of the bone tissue in mice (Starling, 2020).

Tasks of thermal ablation are to significantly decrease (–20…–40 °C) or increase (> 60 °C) the temperature inside the bone tumour in order to kill cells and cause necrosis of the tissue (Moynagh et al., 2018). Minimizing the risk of hyperthermal trauma of nerve cells and other neighboring tissues in the temperature of > 50 °C requires passive or active strategies of thermal protection. Furthermore, nerve elements cannot feel temperature higher than 45 °C, which leads to damage to nerves and paralysis or paresis in some regions (Tomasian et al., 2020). The mechanism of effective destruction of the tumour is based on coagulation necrosis of the bone tissue under the influence of temperature > 50 °C to the cytotoxic level (Goldberg et al., 2000; De Tommasi et al., 2021). Currently, effective means of hyperthermia are considered to be radiowave, laser and microwave methods of destroying the tumour cells without physically removing the tumour (Cazzato et al., 2021).

Mild thermal stress increases the processes of regeneration of blood vessels and bones, though the mechanisms of angiogenesis and osteogenesis have not been studied. A group of scientists recorded enhanced development of the structures similar to microvessels in the shared cultures of olfactory ensheathing cells (OECs) and primary osteoblasts (pOBs). In the experiment, the shared culture of cells was exposed to mild thermal stress (41 °C, 1 hour), twice a week during 7–14 days. After thermal action in cells of real time polymerase chain reaction, expressions of growth factor of the vessels' endothelium, angiopoietin 1, angiopoietin 2 and necrosis factor of alpha tumour increased. Intensification of thermal shock proteins was found at the level of mRNA and protein in shared cultures. Thermal influence increased the expression of mitogen-activated protein kinase, interleukine-6 and bone morphogenetic protein 2, related to thermal shock proteins. Thermal stress proteins regulated the process of development of new blood vessels and the bone tissue (Li et al., 2014).

In the region of the fracture, temperature rises. Heat and cold greatly influence growth and bone reconstruction. According to the level of influence on the growth processes, temperature is only inferior to oxygen accessibility factor. In the late XX century, growth of bones of newborn animals in length and width was observed to intensify if temperature was over 1.5–3.0 °C. In the experiment, tails of baby mice that had been growing at the temperature of 33 °C had greater growth rates and grew longer than in 8–21 °C. Furthermore, the babies in the warmer environment matured earlier. No significant changes were observed in length of lumbar and first three tail vertebrae that – unlike distal vertebrae – are warmed by the body of animals regardless of the ambient conditions (Al-Hilli & Wright, 1983a). In a modified experiment, tails of mice that had grown in 33 °C temperature were exposed to radiation (5,000 rad). Such a fast growth of tails was observed for 12 hours, but then the growth stopped (Al-Hilli & Wright, 1983b).

After multiple transitional or single long exposures to hyperthermal conditions (39–41 °C), the ability of stromal cells of the bone marrow to mineralize significantly increased. On the other hand, prolonged influence of low temperatures (33 °C) inhibited the mineralization of cells of line of osteoblasts (Beresford et al., 1993). Last year, an experiment was conducted in a Finnish sauna which comprised 12 sessions, 3 procedures a week. It included 5 sets of 10 min-long heat influence in 100 °C and the moisture of 20–30% with 5 min break for recovery in 22 °C temperature and 40–50% moisture. During the sessions of heat influence, the participants drank water ad libitum (300–500 mL). Finnish sauna thermotherapy led to improvement of the parameters of muscular and tissue masses. In particular, muscular weight of the legs increased by 1.07%, mineral density of the bone tissue by 7.70%, and content of bone mineral by 6.17%. Increase in muscular weight was observed after six sessions at a Finnish sauna, which was higher than after twelve sessions (Toro et al., 2021).

A study reported that heat stress effectively induced the development of new bone tissue in an experimental model with 58 rats and 10 rabbits. The experimental animals underwent hyperthermia in 45 °C for 15 min once and three times a week, the changes in their bone tissue being determined by x-ray and histological evaluation. After the procedure of thermotherapy (1 time/week over 4 weeks), the experimental groups of rats and rabbits were observed to have a heightened level of osteogenesis compared with the control. The researchers inferred that thermal stimuli with heated materials enhance osteogenesis and increase the area of the development of bones and would be useful in treatment of bone defects in cases of skeletal diseases (Ota et al., 2017).

One side of the body of 20 young mice was heated by the temperature of 40 °C for 40 min/day for 14 days after weaning. Increments were seen in the lengths of ear (8.8%), posterior part of the foot (3.5%), femur (1.3%) and lower limb (1.5%) on the body sides that had been exposed to heat. Rates of elongation of the heated lower limb – compared with the control – were 12% higher (15 μm/day). Seven weeks after the study, the animals were tested once again to check the retention of the growth rates among the experimental animals. In adult animals, the ears, femurs and lower limbs remained 6.0%, 3.5%, 1.0% longer on the heated side. On the other hand, no differences between the left and right sides of the bodies of the control animals were found. Those results exclude the effect of factor of natural side asymmetry (Serrat et al., 2015). Physiological changes in rodents that had been kept in cages at the standard temperatures (20–26 °C) were related to "cold stress" and comprised changes in metabolism, cardiovascular parameters, breathing and immunological functions (Hankenson et al., 2018).

Sublethal thermal shock (42 °C, 1 hour) significantly inhibited proliferation of three lines of cells of osteosarcoma – HOS85, MG-63 and SaOS-2. It decreased the activity of alkaline phosphatase in all three cellular lines. The indicated enzyme influenced the processes of deposition of calcium in the bone tissue. It is considered an absolute marker of activity of osteoblasts that provide bone growth. Inhibiting action of sublethal heat shock toward the activity of human osteocarcinoma cells may be used as a possible additional therapy in treatment of patients suffering from bone cancer (Trieb et al., 2007).

Resorption of the bone tissue is a part of the normal physiological metabolism. The process of remodeling of the bone tissue occurs in several phases: activation, resorption and growth (osteogenesis). Specific biochemical markers of bone resorption include enzyme of osteoclasts – tartrate-resistant acid phosphatase, and markers of their growth – enzyme alkaline phosphatase. Tartrate-resistant acid phosphatase is one of the isoenzymes of acid phosphatase. It is synthesized in osteoclasts and released by them into the extracellular environment during the resorption of the bone tissue. The influence of local heat on the formation of new bones in rats was studied on 64 males with expanded sagital suture on the skull. It was exposed to infrared light for 20 min a day over 5 days. Temperature was measured by biothermometer slightly above the suture. The experimental animals were divided into four groups according to increase in the temperature: the control, and groups of 1.0, 2.5 and 4.0 ℃ increases. The following results were observed: 1) in the control group and group of 1.0 ℃ increase, the suture tissue was observed to have insignificant reaction of acid phosphatase, except the bone marrow and neighbouring region of capillary vessels; 2) in the region of the suture in groups of 2.5 and 4.0 °C increases, a growth of newly formed bone was found. The obtained results indicate that the extent of the development of the bone tissue depends on the temperature of the corresponding tissues (Ogawa, 1990). When cartilage of rat's tail had been exposed to the influence of temperature from 41 to 46 ºC, its growth was insignificantly inhibited and underwent necrosis. The authors indicate the reason for direct thermal damage being denudation of protein (Morris et al., 1977). In the experiment on rabbits' femur, the rabbits were exposed to microwaves for 45 min once/twice a week. The temperature on the surface and inside the femur was within 42.5–44.0 ºC. Following four-six procedures, the bone was cut out for histopathological study. There were found numerous cement lines and a small number of osteoblasts and osteoclasts that paved trabecular surfaces. The indicated changes suggested that the process of remodeling of bone in the conditions of hyperthermia increased compared with the control. In bone exposed only to hyperthermia (without surgical trauma), no such changes were observed (Leon et al., 1993).

In an experiment, 68 newborn female mice were kept in low (16 °C) or warm (25 °C) temperatures. Some groups of animals were performing exercises on a running wheel. The study revealed that exercises alleviated the effect of low temperatures on the process of elongation of the limbs after 11 days of physical load. Regardless of the temperature, all runner mice had elongated limbs, and individuals that had performed no exercises had shorter limbs. The authors concluded that the effect of physical exercises affected exclusively the limb bones and was not a systemic endocrine reaction (Serrat et al., 2010).

Heat shock proteins and other factors of regulation of the bone tissue

Heat shock proteins (HSP) are a class of proteins formed after increase in the body temperature. Rates of their formation are regulated at the stage of their transcription under the influence of heat shock factor (HSF). Based on their molecular masses, mild stress proteins are classified into HSP-72, 70, 60, 10 and others having the values of 72, 70, 60, 10 kDa respectively. In the late XX century, the mechanism of their action was determined to be based on the regulation of renaturation and intracellular transport of proteins damaged by high body temperature or other factors. Functionally, they take care of other proteins, providing them with normal operation of functionally active regulators, catalysers, transporters, etc., and utilizing depleted and irreversibly damaged protein molecules (Kuibida et al., 2021).

Acute and chronic influence of heat improves metabolic functions in the organism using increase in the activity of membrane enzyme of adenylyl cyclase, which transforms ATP into cAMP and corresponding protein kinase which phosphorylates proteins (Kostromin et al., 1984; Hafen et al., 2018). Protein kinase acts as a sensor that detects changes in AMP/ATP ratio (Hasenour et al., 2013). During decrease in ATP, the activity of protein kinase increases (Kostromin et al., 1986; Demidov et al., 1991). This results in intensification of metabolic pathways of ATP formation as a result of increase in glycogenolysis and lipolysis (Herzig & Shaw, 2018). Even one session of heating for ~ 2 hours may increase the activity of protein kinase and the process of phosphorylation (Hafen et al., 2018).

Heat influence induces HSP expression. They stabilize proteins that strengthen the transmission of the signals of nitrogen oxide (NO), decrease oxidative stress and inflammation of the vessels and improve their function (Brunt et al., 2016). During passive heat stress, blood circulation in the legs increased ~ 3–4-fold (Leicht et al., 2019). Vessel network is of essential value for transportation of regulators that support processes of endochondral ossification. Bone has a dense capillary network, and the signal molecules that participate in its growth must overcome the cartilage. The cartilage is included in the epiphyseal plate, but it has no penetrating blood supply (Racine & Serrat, 2020).

The cartilage has no blood vessels and its nutrition is performed by diffusion of substances. The semi-penetrable "barrier at the border of vessel-cartilage surface" obstructs the molecular transport. To study the peculiarities of overcoming this obstacle, a model of heating the hind legs was used for manipulating the blood circulation in bones in 5-week-old female mice. In the experiment, dextrans were used weighing 10, 40 and 70 kDa, which are close in size to physiological regulators. Increase in the temperature in hind legs from 22 to 34 °C led to increase in vascular access of the abovementioned molecules. In 34 °C, penetration of dextrans 10 kDa into the growth plate increased by > 150%, and 40 and 70 kDa increased only by < 50% (Serrat et al., 2014).

Lengthwise growth of bones is regulated by primary endocrine factors that perform coordinated or single actions. They include: growth hormone, insulin-like growth factor I, estrogens, androgens, group D vitamins and others (Wang et al., 2010; Lui et al., 2014). Elongation of bones is a complex process caused by several internal factors (hormones, growth factors) and external variables (nutrition, environment). Insulin-like growth factor I (IGF-1) is the main hormone that stimulates the development of the bone tissue (ossification) and a drug for treating skeletal diseases in children. Temperature directly affects the growth through changes in IGF-1 expression (Racine & Serrat, 2020).

Increase in IGF-1 expression in skeletal muscles of broiler chickens was recorded at heightened temperatures during rearing (Al-Zghoul et al., 2016). Heat manipulations heightened the levels of mRNA heat shock proteins (Hsp70 and Hsp60) and heat shock factors (HSF3 and HSF4) in muscles of birds during the first week of life and during heat stress. On days 14 and 28 after weaning, the basal levels of mRNA Hsps and HSF of chickens were significantly higher than in the control (Al-Zghoul, 2018). During the heat stress and after weaning of broiler chickens, basal and dynamic expressions of mRNA heat shock proteins (HSP108 and HSP90) and heat shock factors (HSF-1 and HSF-2) increased as well (Al-Zghoul & El-Bahr, 2019). Similar results were obtained when growing eels. Fish grown at 22 °C temperature were longer and had increased expression of IGF-1 gene than those grown in 16 °C (Politis et al., 2017). Moreover, increased temperature weakened the affinity between IGF-1 and its acid-labile subunit (ALS), leading to increase in free and active IGF-1 (Holman & Baxter, 1996).

Insulin-like growth factor I is mostly synthesized in the liver under the influence of growth hormone, and is also produced by bones and muscles. A group of researchers determined positively significant relationships between IGF-1 concentration and levels of albumin, bone alkaline phosphatase and creatinine kinase, respectively. They came to the conclusion that IGF-1 levels in blood serum are associated with the concentration of markers of formation of skeleton muscles and bones, but not the markers of bone resorption during general physical activity in healthy adults (Lee et al., 2021).

A direct effect was found of temperature on stromal cells of human bone marrow and cells similar to human osteoblasts, obtained from the osteosarcoma culture. It was revealed that both types of cells had high tolerance to long exposure to mild thermal shock (1 hour in 39–41 ºC). The concentration of heat shock protein HSP70 was observed to increase under the influence of hyperthermia (39 ºC) and decrease in 33 ºC. They have come to the conclusion that mild thermal shock induces proliferation, activity of alkaline phosphatase and mineralization in stromal cells of human bone marrow and cells of Mg-63 *in vitro*. By contrast, diathermy of deep layers of the bone tissue by high-frequency alternating current in vivo stimulated its formation and growth (Shui & Scutt, 2001).

A team of authors revealed that HSP70 (200 ng/mL) increases the activity of alkaline phosphatase and promotes the mineralization of the bone tissue. In the conditions of osteogenic induction, this heat shock protein increased the expression of osteospecific genes such as transcriptional factors of family runt Runx2 and osterix (OSX). The authors came to the conclusion that HSP70 promotes osteogenesis and may be a therapeutic mean for treating uncoalesced bones (Chen et al., 2015). Thermal stimuli (40 °C/15 and 45 °C/15 minutes) promoted significant osteogenesis of tibias of rats and rabbits. In the regime of hyperthermia, an increase was observed in biosynthesis of heat shock protein HSP70, activity of alkaline phosphatase and expression of genes related to the bone tissue (Ota et al., 2017), and HSP60 stimulated bone resorption (Hang et al., 2018).

A review has described different stages of the mechanism of remodeling and formation of the bone tissue. It mentioned that the synchronic remodeling of bones includes the resorption and its formation. It comes through a number of stages: 1) bone calm, 2) activation, 3) bone resorption, 4) reversion, 5) formation, 6) stop. Excess of glucocorticoids negatevely affects the molecular signal transmission, induces the apoptosis of osteocytes and osteoblasts by various means and prolongs the life cycle of osteoclasts. Moreover, biosynthesis of biologically active growth factors decreases, leading to decrease in the expression of osteoblasts by molecules that form osteoid. Increase occurs in the expression of mineralization-inhibiting proteins that inhibit the mineralization of osteoids and simultaneous local de-mineralization of the matrix (Povoroznyuk et al., 2021).

In our opinion, the indicated effects of the glucocorticoids on osteogenesis are important for the nutrition tactics of endurance athletes and trauma prevention. Protein nutrition before competitions or during ultratriatlon (8–9 hours of continuous load) increases the processes of glucogenesis and secretion of cortisol. Long excess of cortisol concentration during training leads to increase in the frequency of fractures and other negative consequences, but this problem should be considered separately.

Osteochondral interface between the bone and the cartilage allows these tissues to "communicate" with one another and exchange signal and plastic molecules, thereby providing integrated response to mechanical and thermal irritators (Oliveira Silva et al., 2020). There is a functional connection between the inhibition of the markers of effective bone remodeling following fracture and increase in oxidative stress (Falfushynska et al., 2019). The peculiarities of the influence of modulators of signal molecules, particularly chaperones and other functional proteins, in the conditions of heat stress has been studied (Rakhmetov et al., 2016), and the functioning of active oxygen species as "secondary intermediaries" in the regulation of intracellular signal cascades has been analyzed in the review (Drobot et al., 2013). Furthermore, when the pool of active oxygen species exceeds the antioxidant ability of the organism, the existing antioxidant pathways are undergirded by the synthesis by protective heat shock proteins (Smolka et al., 2000; Archer et al., 2017).

Based on the analysis of theoretic and experimental data, we proposed a model of the mechanism of influence of mild heat shock on bone growth (Fig. 1).

To obtain the full picture of the mechanism of heat influence on osteogenesis, we should proceed from the fact that light heat shock ≈ 39–41 ℃ is a type of stress. It is accompanied by a known ensemble of hormones and factors. Stress provokes the universal secondary messenger – the system of cyclic nucleotides. Membrane enzyme adenylyl cyclase, or ATP pyrophosphate-lyase (cycling) (KF 4.6.1.1), catalyses the transformation of ATP into 5'-3'-cyclic adenosine monophosphate, or cAMP. At the same time, ratio of AMP/ATP in the cell changes. Increased concentration of cAMP activates the cAMP–protein kinase (KF 2.7.11.1). Protein kinase A performs the phosphorylation of chromatin, transcription factors of heat shock proteins (HSF-1, HSF-2, HSF-3, HSF-4), stress proteins (HSP-60, HSP-70, HSP-90, HSP-108) and enzymes of energy-generating processes of glycolysis and lipolysis. Stress protein HSP70 activates alkaline phosphatase and promotes the process of mineralization of the bone tissue. Syntheses of insulin-like growth factor-1 (IGF-1), mitogenic action factors, signals of intensification of blood circulation (NO) and somatotropin in the cell activate. Thermotherapy decreases the affinity between insulin-like growth factor I and its acid-labile subunit (ALS). This leads to increase in free and active IGF-1. Against the background of acceleration of capillarization process, the energy generation and concentration of stimulators of growth of the bone tissue, there occurs activation of functional activity of producer cells – osteoblasts and osteocytes, and the bone elongates (Fig. 1). We see parallels in the mechanism of stimulation of the growth of the bone tissue, increase in the mechanostat of bones exposed to mild heat shock and plyometric means of physical pressure (Kuibida et al., 2021).

Thermoregulation: role of tails and other peripheral body parts

In the sphere of studies of thermoregulation, the body is conditionally divided into two regions: 1) external membrane (skin) – the temperature there fluctuates together with the environment and 2) the internal nucleus (internal organs) – has relatively stable temperature. Increased body temperature in living organisms occurred as a secondary consequence of high rates of metabolism. It is necessary for long functioning of the organism or establishing new ecological niches. Reverse response occurs if physical exercise or other factors increase the heat generation and the internal temperature rises. The body temperature is regulated by physiological and behavioral types of mechanisms. The main physiological reactions to cold are thermogenesis of brown fat tissue, shivers in skeleton muscles which produce heat and cause narrowing of blood vessels, preventing heat losses. By contrast, the action of heightened temperature inhibits thermogenesis, increases perspiration and enlarges blood vessels. Animals use different strategies to achieve the same physiological effect. In high temperatures, the vessels of the body surface enlarge earlier than perspiration starts. Cooling through perspiration causes losses of water, vitamins, and chemical elements. Perspiration against overheating activates when the capabilities of the first stage become depleted. Similarly, the cold first activates vasoconstriction, and then – shivering or thermogenesis. It depends on relative energy expenditures of each mechanism. Thermogenesis is also triggered by various pyrogens: hormones of the pancreas, iodine, bacterial lipids, which induce the production of prostaglandin E_2 and others by endothelial cells (Tan & Knight, 2018). If the mice are kept in the ambient temperature of 4 °C, the warming of the body by thermogenesis uses around 60% of the overall energy (Abreu-Vieira et al., 2015).

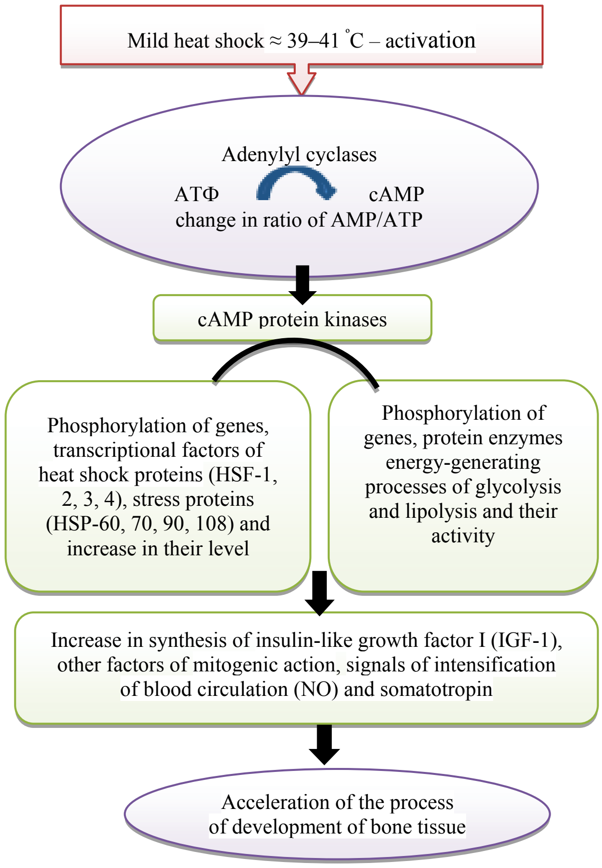

Fig. 1. Role of heat stress proteins in the mechanism of adaptation to heat

Open regions of the body may lose significant amounts of thermal energy if the ambient temperature drops below thermoneutrality. Therefore, there are various mechanisms to prevent heat losses. An effective strategy is based on narrowing of the diameter of the blood vessels (vasoconstriction). It limits the blood flow to the distal body parts and therefore promotes retention of heat (Tan & Knight, 2018). Protection of the organism against heat loss in Cetaceans is carried out by anti-leakage heat exchangers (Heyning, 2001; Davenport et al., 2015). In rats, the key role is played by a mechanism based on vasodilation (Dawson & Keber, 1979; Young & Dawson, 1982). Tails of mice have insignificant effect on their thermoregulation (Škop et al., 2020a), while tails of beavers, muskrats, foxes, rabbits and many others have significant effects. The fall in the ambient temperature triggers functions that provide outflow of heat from tails and they stop functioning as radiators (Stryjek et al., 2021).

A study described the vascular structures in the tail of Florida manatee (*Trichechus manatus latirostris*) which provide anti-leakage heat exchange and retain the thermal energy in the organism. The authors noted that around 1,000 arteries are near 2,000 veins in the cranial end of the caudal vascular bundle. Their number decreases caudally, but the arteries / veins ratio continues to equal 1:2. Close contact causes blood to give heat to cold venous blood and it returns the heat back to the nucleus of the body when the water temperature is low. By contrast, when the animals were in warm marine water, their tails lost heat while their temperatures were lower than of the environment (Rommel & Caplan, 2003). Cold stress syndrome annually affects the manatees in Florida during intense or prolonged cold weather, which is one of the threats to those animals. The thoracic flippers and axillae were noted by the authors as regions of the greatest heat exchange (Erdsack et al., 2018).

In thermal physiology, mice are considered a useful model object to study human diseases. During drastic changes in the environmental temperature from 19 to 39 °C, the mice the tails of which had been amputated and the control specimens had almost the same body temperature and rates of metabolism. Losses of heat through tail were insignificant, but also were useful biomarkers of enlargement of the vessels and thermoregulation. In those experimental individuals, the tail of mice provided only 5–8% of heat emission from the entire body, and in rats this parameter equaled 17% (Škop et al., 2020b). Laboratory rats were subjected to the influence of the ambient temperature ranging 12 to 35 °C according to dry thermometer for ≈ 5.5 h. The same procedure was performed with rats that were kept with a constant temperature around the tail and dead animals. The study resulted in the conclusion that the rat's tail – according to its operation regime – looks like a controller with on/off (activation/deactivation) functions (Dawson & Keber, 1975).

Rodents and other animals prevent heat loss using a broad spectrum of behavioural adaptations: hiding in burrows and nests, sitting on tail, curling into ball, group contact warming. A new thermal adaptation to cold stress has been described as "tail belt" of rodents (*Apodemus flavicollis* and *A. agrarius*). During cold stress, the animals lifted and bent the tail medially and then put it on the spine, the medial rump during eating to prevent heat loss (Stryjek et al., 2021). Mice that had grown up in a cold environment grew much shorter tails (Gordon et al., 2014).

A study focused on the influences of various temperatures on growth of bones of wild male mice. The animals were divided into three groups according to the criterion of different temperatures of their maintenance: 1) thermoneutral, 26 °C, 2) standard, 22 °C, 3) cold, 20 °C. The experiment lasted for 3, 6, 12 weeks with ad libitum access to food and water. The study revealed that the effect of cold was decreased by activation of protein synthesis that broke down the oxidative phosphorylation with respiration in mitochondria of brown fatty tissues. This resulted in increased thermogensis without shivering. After the end of the experiment, the animals of the "cold" group had a notably lower volumetric share of the trabecular bone of the distal part of the femur, thickness and density of the contact and the area of the cortical bone of the lower middle of the femur, compared with mice that lived in 26 °C conditions. The authors came to the conclusion that the low temperature decreased the bone weight in mice (Robbins et al., 2018).

We consider that in the conditions of low temperatures, the ratio of white and brown fat can have an effect on growth of bones. In cold, brown fat warms the body by increasing thermogenesis and synthesis of heat shock proteins. Thus, brown fat compensates the lack of low atmospheric temperature. Prolonged cold and physical loads create moderate stress effect and decrease the reserves of white cold fatty tissue and increase brown warm fatty tissue. Heat speeds up the processes of growth of the bone tissue. This presumption has been confirmed by the results of the experiment conducted by the Dutch scientists. In the experiment, they divided mice into two groups according to their maintenance conditions – individual and social. They observed that after weaning, individual maintenance of six weeks-old male mice C57BL/6J led to white obesity and impeded growth (Schipper et al., 2020). For quantitative evaluation of morphology, rats' bodies from birth to adulthood were kept in cold (17 ºC), moderate (25 ºC) and warm (33 ºC) environments. Compared with animals of moderate maintenance, the rats of "cold" group had shorter ears, tails and lower ratios of tail length to body length. By contrast, animals in warm premises had lower weight, shorter bodies and the greatest ratio of tail length to body length (Villarreal et al., 2007).

Under the influence of heat, the cardiovascular function improves by enlargement of the diameter of blood vessels. Endothelium causes release of vessel-enlarging molecules (nitrogen oxide, histamine), arterial vasodilation, decrease in artery stiffness, modulation of vegetative nervous system, favourable changes in circulating lipid profiles and systemic arterial pressure (Laukkanen et al., 2018).

Elaborated interpretation of ecological Allen's rule

We studied the amplitude of changes in tail length in adult male mammals depending on temperature regime of place of birth. The researchers noted different temperature conditions in broods in deep and surface burrows, hollows, nests, houses and agricultural premises of people for newborn animals. Some rodents Rodentia and Eulipotyphla raise their young in the upper layer of soil where the temperature is lower in spring and summer than in the atmosphere or soil surface. Inertia in the dynamics of changes in the soil temperature increases with depth of its layer. At the depth of 3.2 m, the temperature of soil in the conditions of Ukraine changes in the range of 7 °C over year. Every soil type is characterized by its range of temperature fluctuation at the depth of 20 cm. The average temperature at this depth in the warm period ranges within 6–10 °C for podzolized soils, and 11–15 °C for chernozems. By contrast, in 2020, the average monthly air temperature was within 16–17 °C in western and eastern Ukraine, and 15–16 °C in the northern oblasts, 17 °C in central oblasts, and 18 °C in southern oblasts.

To evaluate the results of tail growth, the elongation index was calculated – ratio of tail length to body length of mammals depending on the place where the newborn were raised. Tail length C (Cauda) is distance from the rectum to the tail end without end hair, and body length L (Longitudo) – from nose end to rectum on the abdominal side; in large animals – from tail end to the base of tail on the back side. The morphological parameters of body and tail lengths were taken from the guides (Migulin, 1938; Reichholf, 2002). Scientific names of mammals are given according to the Commission of Zoological Terminology named after I. I. Schmalhausen and the Ukrainian Teriological Society of the National Academy of Sciences of Ukraine.

Based on the data analysis (Table 1), we distinguished three groups of animals with different parameters of tail growth depending on places where the newborn grew. In the group of short-tailed animals, the index of tail elongation equaled 7.8–7.2, and therefore the rates of functioning of the growth plate of the tail vertebrae of babies in deep burrows in cold regions were low. Representatives of mammals of temperate climate which raise animals in burrows were identified to group of average-tailed animals. Parameters of indexes of tail elongation in this group of animals ranged within 5.4–3.2. The indicated index in the group of long-tailed animals ranged within 2.1–0.7. Functional activity of their growth plates of tail vertebrae was high because the offspring had developed in nests on stems of plants, holes, residential buildings of humans, economic facilities, non-deep burrows with sufficient amount of heat. It should be noted that different ecological living niches and ways of moving of animals also had effect on the intensity of distal growth of the bone tissue. Evolution-caused factors of tail elongation are also its functions as "engine" and "stabilizer" of flight in the processes of swimming or jumping, etc.

According to the ecological-morphological rule (law) of Allen, warm-blooded animals in cold climate conditions have shorter body parts than the same species in the regions with warm climate for the purposes of decreasing heat leakage (Allen, 1877). In our opinion, it would be incorrect to consider Allen's rule as an ecological-geographic pattern, therefore different species may live within one geographic territory and have different morphological parameters, particularly, the brown rat *Rattus norvegicus* and black rat *R. rattus* (C/L index of 1.2 to 0.9, respectively), or birch mouse, mice and voles (Table 1). This ecological-morphological pattern occurs based on different temperature conditions of growth of the bone (limbs, tails) and cartilage (ears) tissues in young while developing.

Conclusion

Mild heat stress increases the processes of growth and regeneration of bones, but the mechanisms of osteogenesis have not been studied in details. We distinguished five zones of temperature influences on the bone tissue with different biological effects: a) the below-threshold thermal zone of <36.6 ºC, insufficient amount of heat is a limiting factor of osteogenesis; b) the zone of the normal temperature ≈ 36.6–37.5 ºC, in this temperature range the processes of breakdown and bone formation are balanced; c) the zone of mild heat shock ≈ 39–41 ºC, the functional activities of osteoblasts, osteocytes and development of the bone tissue increase; d) the zone of sublethal heat shock ≥ 42 ºC, the bone growth is being inhibited; e) the zone of necrotic shock ≥ 50 ºC, the cells of the bone tissue die.

Table 1
Ratios of tail length to body length of mammals depending on places where they grew

Species name	Body length (L, Longitudo) and the average body length (cm) – line below	Tail length (C, Cauda) and average tail length (cm) – line below	Ratio of average tail length to average body length c/L	Location of newborn	Note
Norway lemming *Lemmus lemmus* Linnaeus, 1758	13.0–15.0; 14.0	1.5–2.0; 1.8	7.8	in burrows	common in Scandinavia, burrows are not deep
Podolsk mole-rat *Spalax zemni* (Erxleben, 1777)	23.0	≤ 3.0	7.7	burrows at the depth of 13–21 cm, and nesting chambers at 2.75 m	stay underground almost throughout their life
Lesser mole-rat *Spalax leucodon* Nordmann, 1840	18.5–27.0; 22.8	≤ 3.0 ≤ 3.0	7.6	nesting chambers in burrows at 1.5–3.5 m depth	in April, females give birth to babies that begin living on their own in May
Arctic lemming *Dicrostonyx torquatus* (Pallas, 1778)	12.5–16.0; 14.3	2.0 2.0	7.2	in burrows	settles on slopes and water divides in tundra
Common hamster *Cricetus cricetus* Linnaeus, 1758	21.0–28.0; 24.5	3.0–6.0; 4.5	5.4	in burrows	burrow is down to 2 m deep
European mole *Talpa europaea* Linnaeus, 1758	12.0–16.5; 14.3	2.0–4.8; 3.4	4.2	in burrows	burrow is about 0.5 m deep
Bobak marmot *Marmota bobac* (Muller, 1776)	61.0	≤15.0	4.1	in burrows at 2–3 m depth	winter burrows at the depth down to 5–7 m from the surface
Alpine marmot *Marmota marmota* Linnaeus, 1758	50.0	15.0	3.3	in burrows	lives in mountain meadows and pastures
European ground squirrel *Spermophilus citellus* Linnaeus, 1758	20.0–22.0; 21	6.0–7.0; 6.5	3.2	in burrows	burrows down to 2 m deep
Snow vole *Chionomys nivalis* (Martins 1842)	12.0–14.0; 13.0	5.0–7.5; 6.3	2.1	in burrows	inhabits mountainous and rocky terrain
Bank vole *Myodes glareolus* (Schreber 1780)	8.0–12.0; 10.0	3.5–7.8; 5.7	1.8	in spherical nests underground	in summer and sometimes in warm period constructs nests in burrows
European water vole *Arvicola amphibius* (Linnaeus, 1758)	16.0–22.0; 19.0	10.0–15.0; 12.5	1.5	in burrows	skillful swimmer
European pine vole *Microtus subterraneus* (Selys Longchamps, 1836)	7.5–10.5; 9.0	2.5–3.9; 6.4	1.4	in burrows	lives in old ravine forests
Muskrat *Ondatra zibethicus* (Linnaeus, 1766)	26.0–40.0; 33.0	20.0–27.0; 23.5	1.4	in burrows	entrance to the burrow is underwater
Striped field mouse *Apodemus agrarius* (Pallas, 1771)	9.0–12.2; 10.6	6.6–8.8; 7.7	1.4	in burrows	burrows at small depth
Garden dormouse *Eliomys quercinus* (Linnaeus, 1766)	11.5–15.0; 13.3	9.4–12.1; 10.8	1.2	in nest on trees	settles mostly in spruce-beech forests
Brown rat *Rattus norvegicus* (Berkenhout 1769)	20.0–28.0; 24.0	17.0–23.0; 20.0	1.2	in natural conditions, they dig simple burrows at the depth down to 50–80 cm near water bodies	in houses, prefers basements; good at swimming and diving (can be up to 3 days in water)
Edible dormouse *Glis glis* (Linnaeus, 1766)	13.0–18.0; 15.5	11.0–15.0; 13.0	1.2	in tree holes, nests of birds, and also under roofs of houses	breeding season from late June to mid August
Hazel dormouse *Muscardinus avellanarius* (Linnaeus, 1758)	7.0–9.0; 8.0	6.0–7.5; 6.8	1.2	in rounded nest on branches of trees, shrubs or small hollows	wakes up from winter hibernation when the environmental temperature is higher than 20 ºC
Forest dormouse *Dryomys nitedula* (Pallas, 1779)	8.5–12.0; 10.3	6.0–11.3; 8.7	1.2	in hollows of trees, bird houses, agricultural premises	often settles near people, particularly in agricultural premises.
Harvest mouse *Micromys minutus* (Pallas, 1771)	5.5–7.5; 6.5	5.1–7.2; 6.2	1.0	spherical nest are constructed on stems of herbaceous plants at the height of 40–100 cm	no entrance to the nest. Every time the female makes a new hole and then closes it
House mouse *Mus musculus* Linnaeus, 1758	7.0–10.0; 8.5	7.0–10.0; 8.5	1.0	in natural conditions, lives in burrows: at 20–30 cm depth in the warm season	can live anywhere in peoples' houses
Western wood mouse *Apodemus sylvaticus* (Linnaeus, 1758)	7.5–11.0; 9.3	7.0–11.5; 9.3	1.0	in burrows	skillfully climbs trees and shrubs
Yellow-necked mouse *Apodemus flavicollis* (Melchior, 1834)	8.5–13.0; 10.8	9.5–13.5; 11.5	0.9	in burrows and holes of trees	skillfully climbs trees and shrubs; good at jumping and moves across territories
Black rat *Rattus rattus* Linnaeus, 1758	15.0–23.0; 19.0	17.0–25.0 21.0	0.9	in natural conditions, it does not usually dig burrows, but constructs spherical nests or occupies tree holes	heat-loving species. In houses, raises young under roofs to avoid competition with brown rat
Northern birch mouse *Sicista betulina* (Pallas, 1779)	5.8–7.4; 6.6	8.5–10.0 9.3	0.7	constructs nests in hollows, moldering tree stumps, and branches	uses tail as an additional grabbing organ

We determined the most important elements of the mechanism of the influence of mild heat shock on bone growth. Mild heat shock equaling ≈ 39–41 ºC is a type of stress. The stress provokes membrane enzyme adenylyl cyclase and cAMP-protein kinase. Protein kinase A phosphorylates chromatine, transcription factors of heat shock proteins (HSF-1, HSF-2, HSF-3, HSF-4), stress proteins (HSP-60, HSP-70, HSP-90, HSP-108) and energy-generating enzymes of glycolysis and lipolysis. Stress protein HSP70 activates alkaline phosphatase and promotes the mineralization of the bone tissue. Syntheses of insulin-like growth factor-1, mitogenic action factors, signals of blood circulation intensification (NO) and somatotropin in cell increase. Heightened temperature decreases the affinity between insulin-like growth factor I and its acid-labile subunit, leading to increases in free and active IGF-1. Against the background of acceleration of blood circulation process, generating energy and the level of stimulators of the bone tissue, the functional activity of cells that create bone – osteoblasts and osteocytes – increases.

Drawing from the analysis of modern knowledge of the role of heat shock proteins in the processes of increment of bone and cartilage tissues, we have developed and added to Allen's rule. We may state the following: "In warm-blooded animals of the same and different species, distal body parts (tails) are longer if the young after the birth have been developing in the conditions of higher temperature". The indicated tendency is realized to the fullest in the zone of mild heat shock by intensified biosynthesis of heat shock proteins, insulin-like growth factor I and other stimulators of growth processes of the bone tissue. Additional, evolution-caused factors of changes in tail length are its functions as "engine" and "stabilizer" in the process of swimming or jumping.

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