REVIEW

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HEAT SHOCK PROTEINS IN ADAPTATION TO PHYSICAL ACTIVITY

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The review article presents the author's model of one of the blocks of the integrated adaptation mechanism to physical activity and the accompanying moderate heat effects. The participation of heat shock proteins in the stabilization of the tertiary structure and in the restoration of the function of proteins damaged by temperature and physical stressors but performing catalytic, transport, reception or protective role and being involved in the processes of contraction- relaxation and muscle and bone tissue remodeling is discussed.

K e y w o r d s: heat shock proteins (HSP), chaperones, protein folding, physical activity.

Heat shock proteins form a biologically complex extracellular protein network of stress, which has various biological, homeostatic and immunomodulatory characteristics. In addition to heat exposure some other stress factors such as virus infection, cytokines, oxidative stress, ionizing and UV radiation, glucose deficiency, exposure to toxins and some metals stimulate the expression of shock proteins. The shock response of cells is a universal phenomenon in which a part of proteins undergoes the process of denaturation. Denaturized cell proteins require the help of special proteins (chaperones) for renaturation which ends with their return to the previous functionally active state [1]. In the early 60th of the XX century, it was shown that heat shock proteins (HSP) are the class of proteins which are formed due to a rise in body temperature to the threshold level. Regulation of the speed of their formation occurs at the stage of the transcription under the effect of heat shock factor (HSF). Heat shock or stress proteins are classified on the basis of their molecular weights on HSP-72, 70, 60, 10 and other, which have the meaning 72, 70, 60, 10 kDa, respectively. In the 80th of the XX century, first steps were taken to establish the mechanism of their action. It is based on the regulation of the renaturation process and intracellular transport of proteins damaged by a high body temperature. Functionally, they supervise other proteins. Shock heat proteins provide conditions for rapid search for native conformation for unstructured polypeptide chains, participate in stress management and embryogenesis, in the reactions of cellular and humoral immune responses, pathogenesis of most diseases, stimulate osteogenesis etc. They insure normal operation of functionally active regulators, catalysts, transporters etc. and utilize worn-out and irreversibly damaged protein molecules [2].

The heat affects physical exercise and causes mild physiological stress. It is accompanied by an increase in body temperature, blood pressure and heart rate. Glucose performs the role of the main energy source for submaximal physical activity while thermal treatment leads to an increase in its absorption. During passive heat stress there is 3-4-fold increase in blood circulation in the legs [2]. Heat therapy protects skeletal muscles from the insulin-induced resistance to fat [3]. Daily effect of heat stress on mice kept in cages at 40°C, 30 min per day, for 7 days, prevents the loss of mitochondrial content (reduction of ubiquinone-cytochrome), reduction of oxidative capacity (reduction of maximal activity of citrate synthase and 3-hydroxiacyl-KoA dehydrogenase) in denervated muscles [4].

It was found that in the process of performing endurance physical exercise ≈75% energy from oxidation of metabolic fuel and ATP hydrolysis is releases in the form of heat. This leads to a rise in body temperature. In particular, the temperature in

muscles can rise to 40–42°C. Overheating is equated to a damaging thermal stress, while a moderate heat stress enhances insulin sensitivity and the rate of mitochondrial formation in skeletal muscles. Regulation of mitochondrial biogenesis is carried out by the enzyme cAMP-dependent protein kinase. It has been experimentally discovered that after 1-hour of light heat stress at 40°C increases the activity of the indicated enzyme and expression of some regulatory genes of mitochondrial biogenesis [5]. Activation of biosynthesis HSP72 during the prolonged exercise or pharmacological stimulation protects the body from obesity and insulin insensitivity [6].

In the collective work of Tilman Grune, it was reported that the action of chaperone HSP70 is manifested in the first 1–3 h after mild oxidative stress. The stress protein instantly provides an increased ability to break down proteins damaged by reactive oxygen species. Adaptation to mild oxidative stress occurs by increasing proteasome activity and degradation of oxidatively modified proteins within 24 h [7, 8].

Mechanical stress, acidosis, hypoxia, ischemia, formation of reactive oxygen species and changes in calcium signaling independently cause HSP induction [8, 9]. Physical exercise causes heat shock protein (HSP72) to circulate, which precedes increased expression of genes and proteins in skeletal muscle. Increased metabolic stress due to depletion of bioenergetic substrates (glycogen) increases the expression of HSP72 caused by physical activity [10]. A similar effect of enhancing the biosynthesis of 72 kDa heat shock proteins (HSP70) was observed in rats at 60 min of submaximal exercise on a treadmill at a speed of 17 m/min and a slope of 0%. Heat shock protein synthesis predominated after exercise in a high temperature environment (exercise at 32°C), but slowed down in a cold environment (exercise at 14°C) [11]. Therefore, the increase in the level of HSP72 expression after exercise is the result of one and many physiological stressors.

The relationship between the induction of HSP72 and intensity of physical activity of skeletal muscles is described. The experiments were carried out on seven groups of male Sprague-Dowley rats. Different work schemes on treadmill differed in running speed from 15 to 33 m/min. Increased expression of HSP72 occurs during aerobic exercise and speed and strength work. The significantly higher value of HSP72 regulation after exercise in fast-acting type II muscle fiber compared to the slow-acting type I muscle fiber was found. The authors concluded that the speed-power load involves the work of glycolytic fast type II muscle fiber, and this leads to increased synthesis of stress proteins with a large dynamic range [12].

The level of heat shock protein in the skeletal muscles of young and old rats after speed and strength training was studied by Zsolt Murlasits et al. [14]. No differences in mRNA expression were found between the control and experimental groups in the muscles of old and young animals for any of the four genes studied Hsp72-1, Hsp72-2, Hsp72-3 and HSP70. The data are consistent with the hypothesis that higher levels of oxidative stress in the muscles of older animals limit HSP levels and/or function in response to high-intensity contractile stress. Muscle fibers of the second type of young and old animals are able to increase the expression of HSP in response to high-intensity contractile stress [13].

The hypothesis according to which highly active training increases insulin sensitivity much more than moderate is carried out in the work. Physical activity in a wide range of intensity and volume, minimizes insulin resistance, which develops with a sedentary lifestyle. However, the exercise model, which provided ~170 min of exercises per week, improved insulin sensitivity much more than the program, which used about 115 min of exercises per week, regardless of the intensity and amount of exercise. Therefore, the total duration of exercise should be taken into account when developing training programs to improve insulin action [14].

There is a functional relationship between increased oxidative stress and suppression of markers of effective bone remodeling after fracture [15]. The interaction of universal enzymes of peroxiredoxin, which have the functions of signaling molecule modulators, properties of peroxidases and chaperones with other functional proteins under heat stress were studied [16], and the functioning of reactive oxygen species as “secondary mediators” “secondary messengers” in the regulation of intracellular signaling signals [17].

It is proved that the pool of reactive oxygen species exceeds the antioxidant capacity of the body, the existing antioxidant pathways are insured by the synthesis of protective heat shock proteins. Acute cases of physical exercise cause a sharp increase in the level of HSP72 for 24 h, while regular training regimes usually lead to minimal increase in its level after training [18, 9].

A group of researchers conducted experiments on six untrained and six aerobically trained young men. To characterize the stress response, a 45-minute protocol of physical exercises was performed with an intensity of 75% VO_2max. Trained men had significantly higher levels of heat shock protein 60 (HSP60, 25%) and alpha-B-crystalline (43%) at rest phase compared to untrained individuals. The bodies of the trained men perform the selective regulation of basal heat shock and antioxidant protein content and do not show any significant stress response to regular running exercises [19]. A low level of increased stress proteins during endurance physical activity in trained individuals is the result of acquired adaptation to systematic training.

Exercise lasting 5–8 weeks increased HSP72 levels in 40 untrained individuals who performed elbow extension at high and low intensity. The level of extreme responses to exercise was higher after high-intensity exercise, compared with low-intensity exercise. After suspension of physical activity, basal HSP expression in trained subjects returned to the previous level [20].

The body's ability to absorb oxygen is related to the induction of HPS and the flexibility of metabolism. Heat treatment effectively prevents the development of insulin resistance and glucose intolerance in rats using a high-fat ketodiet. The positive metabolic effects of heat shock protein (HSP72), which include increase oxidative capacity and enhanced mitochondrial function, provide the protective effects of thermal shock. A single exposure to heat effect (41°C, lasting 20 min) based on a 3-day ketodiet lead to a decrease in blood glucose and adipocyte size, concentration of triglycerides in the liver and muscles followed by restoration of insulin sensitivity in glycolytic muscles in rat runners of small capacity. High-capacity runners were protected from a high-fat diet [21].

It was stated that muscles composed of slow-acting type I fiber have a high basal expression of HSP72 than muscles composed of fast-acting type II fiber. In contrast, the stress protein content in fiber of type II in soles did not differ from the fibers of type II in extensor muscles of the toes in rats [22].

During and after exercise, Hsp60 provides restoration of mitochondrial proteins together with its helper – co-chaperonin Hsp10. Mitochondrial proteins that enter the inner cavity of the chaperone Hsp60 with the Hsp10 lid are protected from the interaction with metabolites of physical activity interaction with metabolites caused by physical activity. About one-third of the cellular stress protein can be found outside the mitochondria. Membrane-bound and extracellular Hsp60 act as a powerful stimulator of the immune response. In general, it has an immune stimulating and immunosuppressive potential and depends on the context in which the interaction with the cellular network of the immune response is carried out [23, 24].

In addition to its essential role in the lens of the eye, αB-crystallin (HSPB5) retains its main function in striated muscles during physiological and pathological modifications. The effect of one-hour running activity on the HSPB5 content in red and white narrow calf muscles (Gastrocnemius), which contain slow oxidative and fast glycolytic/oxidative fiber as well as on soleus muscles, which mainly consist of slow oxidative fiber types. A significant increase in phosphorylated HSPB5 in skeletal muscles (Gastrocnemius) was found and soles with more slow type I and II/X myofibers [25].

In a study by G. Paulsen et al. eleven healthy men performed 300 maximal eccentric actions involving the quadriceps muscle. Spatial lateral biopsies were collected in 30 min and 4, 8, 24, 94 and 168 h of physical activity. After a 30-minute exercise the level of HSP27 in myofibril fraction increased by 15 times. The level of cytosolic HSP70 increased to 203% of the control level in 24 h after physical exercise. In 4 days the myofibril content of bound HSP70 increased almost 10-fold. Levels of HSP27, alpha-B-crystallin and HSP70 were higher on the first day after physical activity. HSP27 and alpha-B-crystallin seemed to respond immediately to a maximal eccentric exercise and function as stabilizers of disrupted myofibril structures. However, later the general concentration of heat shock proteins especially HSP70 appeared to be higher. Obviously, they do not only stabilize the body homeostasis, but also play a certain role in skeletal muscle recovery and processes of remodeling/adaptation to strength exercises [26, 27].

During 2 and 11 weeks of power training, fifteen young men did one set of lower body exercises and three sets of upper body physical exercises. The biopsies were analyzed for HSP27 content in the cytosolic and cytoskeletal fractions, and HSP70 and αB-crystallin in the cytosolic fraction. In two groups of individuals, the concentration of heat shock proteins was increasing. In contrast, in cytosolic fraction Vastus lateralis after 11-week training HSP27 level increased by 180%, HSP70 by 146%, and αB-

crystallin by 184%. In the trapezoid, an increase in HSP27 was observed in the cytosolic fraction after 2-week training by 149%. However, the trapezoid contained higher levels of HSP70 and αB-crystallin than Vastus lateralis at baseline. HSP27 levels in the cytoskeletal region did not rise significantly in any of the muscles. It is concluded that elevated levels of HSP27, HSP70 and αB-crystallin in cytosol do not depend on the amount of strength training [27].

At rest phase, skeletal muscles in aerobically trained men had significantly higher level of HSP60 +25%) and αB-crystallin (+43%) compared to the untrained individuals due to the high percentage of type I and II fibers. It has been suggested that Hsp60 may be a molecular marker of physiological adaptation to aerobic exercise. After establishing the content of stress proteins, a group of trained men performed 45-minute protocol of physical exercise with the intensity corresponding to 75% VO2max. In trained men physical activity did not activate any stress response to regular running training. There was no significant increase in HSP70, HSC70, HSP60, HSP27, αB-crystallin content in muscles [19].

The relationship between HSP60 and GRP75 expression and skeletal muscle oxidative potential was investigated by assessing citrate synthase activity after endurance training compared with sedentary control. Treadmill training increased citrate synthase activity by 47%, HSP60 expression by 103% and GRP75 by 105%. There was a significant correlation between citrate synthase activity and HSP60 expression in the plantar muscle – Plantaris muscle (a muscle in monkeys necessary for their prehensile lower limbs). These findings are consistent with the hypothesis that an adaptive response to exercise is accompanied by increased expression of HSP60 and GRP75 to support protein import and coagulation [28].

Endurance training resulted in an increase in heat shock protein (Hsp60), amount of mitochondrial copies, and expression of three isoforms of peroxisome proliferation 1-alpha receptor coactivator (PGC1α) in Plantaris muscle type I muscle fibers. The coactivator is used to assess the efficiency of burning fat and carbohydrates, increasing the number of mitochondria and endurance during prolonged exercise. Activation of PGC1α synthesis occurs during the action of various stressors: cold; endurance exercises to accelerate the conversion of lactate to glucose and its return to energy; increasing the content of metabolites, in particular reactive forms of oxygen and nitrogen to neutralize them [29].

Heat induces the expression of heat shock proteins, which stabilize many other proteins important to the body. In particular, these are proteins (Hsp90) that improve the transmission of nitric oxide (NO) signals, reduce oxidative stress and vascular inflammation, and improve their function. To study the effects of heat therapy, V. E. Brunt and his colleagues immersed the subjects shoulder-high in water with a temperature of 40.5°C until their rectal temperature reached 38.5°C, which took about 25–30 min. Then they sat down so that the water reached the waist level for another 60 min (up to 90 min in the bath). The experiment was repeated 4-5 times a week, a total of 36 sessions over 8 weeks. Heat therapy increased the diameter of blood vessels (dilation), reduced arterial stiffness, mean arterial and diastolic blood pressure, the thickness of the internal environment of the carotid artery. The effects of heat therapy were the same or greater than after physical exercise [30, 31].

Based on the literature data analysis, we present a model of one of the blocks of the integrated mechanism of adaptation to physical activity and moderate heat exposure (Fig. 1, 2). Physical exercise includes at least two well-known blocks of the first adaptation system to overcome stress; endocrine mobilization – biosynthesis of adrenaline, noradrenaline, cortisol, glucagon, thyroxine, β-endorphin. Physical work is accompanied by the accumulation of ketone bodies, reactive oxygen species, lactate, lowering the pH of the environment, depletion of the functionality of service systems, bioenergetic substrates and increasing muscle temperature above the threshold value of 38.5°C (Fig. 1). Physical and temperature stressors and their combinations serve as a signal of initiation of the second adaptation system for the continuation of physical work. The concentration of heat shock proteins in muscle fiber increases. They accelerate renaturation processes and stabilize the tertiary structure of other important proteins - regulators, catalysts, transporters, those that perform structural, protective, receptor and motor functions and are damaged by fever or other derivatives of exercise (Fig. 2). As a result of their action, the functional activity and transport of all proteins involved in the processes of muscle contraction and relaxation, remodeling of muscle and bone tissue, etc. is restored. The signalling capabilities of exercise or heat stressors depend on their threshold values. In particular, if the pool of reactive oxygen species ex-

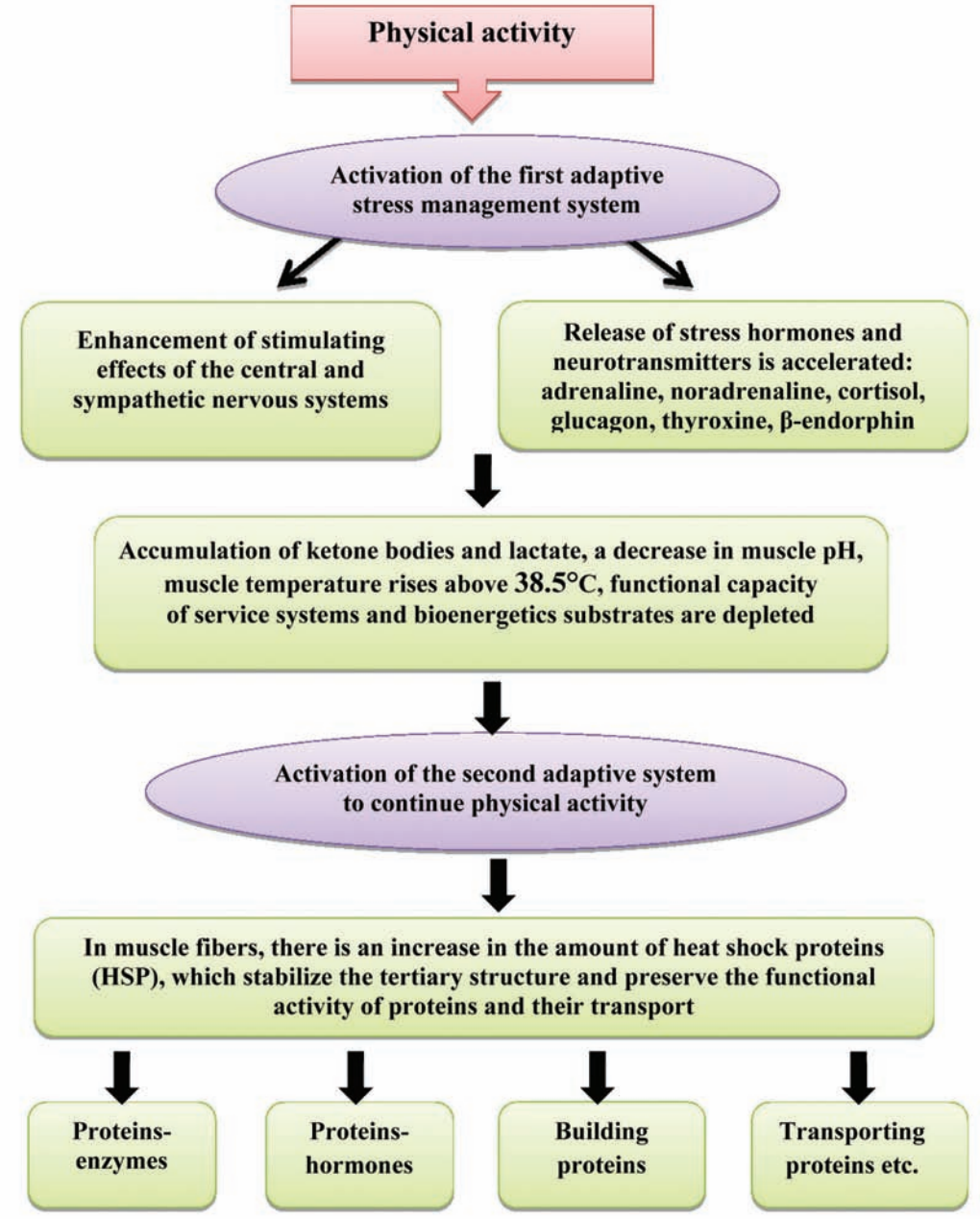

Fig. 1. Role of heat stress proteins in the mechanism of adaptation to physical

ceeds the antioxidant capacity of the body; the oxidative potential of skeletal muscles does not provide physical work in a certain power zone; low level of cAMP-dependent protein kinase activity is not able to maintain the required level of expression of genes that regulate mitochondrial biogenesis and human endurance - in each case, the body insures its own adaptation by synthesizing protective proteins of heat shock (Fig. 2).

Thus, heat shock proteins accelerate the processes of folding of newly formed, restoration of tertiary and quaternary structure of functioning, but damaged by fever or other derivatives of exercise and utilization of worn-out proteins. They optimize membrane transport in the mitochondria and endoplasmic reticulum. These proteins interact with receptors of glucocorticoids and other stress hormones and partially or completely block their effect on biochemical processes that determine the parameters of strength, speed and endurance. Some effects of individual heat shock proteins in physical activity we presented graphically (Fig. 2).

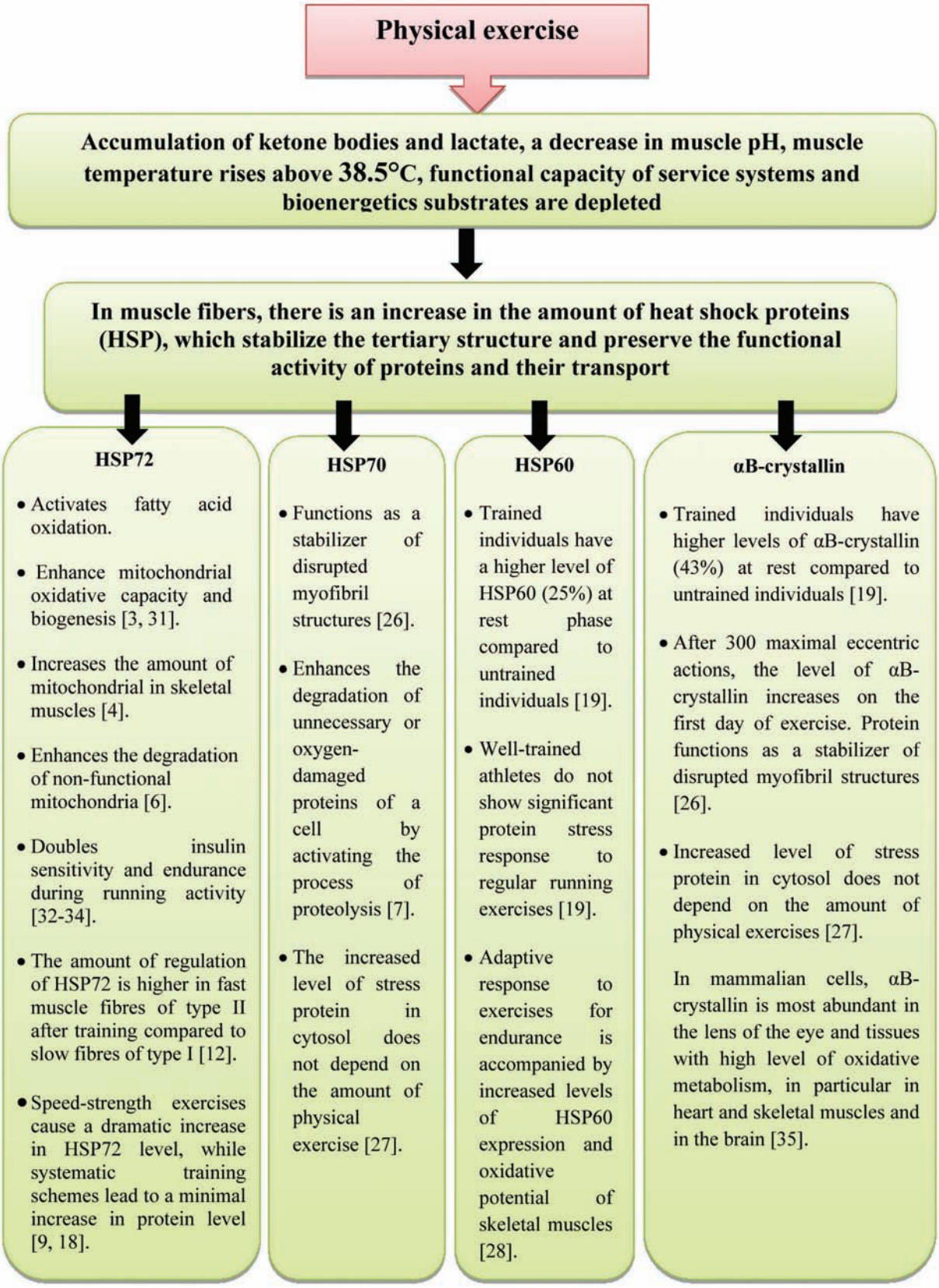

Fig. 2. Effects of heat-shock proteins in physical activity

Valuable results of experimental research and reviews, original ideas about the role of heat shock proteins are found in publications [36, 40, 42, 46, 48, 51, 52].

Conclusions. Mild thermal stress and physical exercise have much in common in their effects on human bodies and animals. The effect of both factors causes moderate physiological stress, accompanied by an increase in blood pressure, heart rate, body temperature and increased expression of heat shock proteins (HSP). Their synthesis accelerates after exercise in high temperatures, but slows down in cool conditions.

Overheating is equated to harmful heat injury, and light heat stress increases the sensitivity of target cells to insulin and their energy reserves, activates the formation of mitochondria and aerobic capacity, proteasomes and the degradation of oxidatively modified proteins and protects the body from obesity.

Increased metabolic stress due to depletion of glycogen or other bioenergetic substrates increases the expression of HSP72 caused by physical exercises. It is the result of not one, but many physiological stressors associated with exercise. The level of regulation by stress proteins is higher in fast type II muscle fibers compared to slow type I fibers after training. The speed-force load involves the work of glycolytic muscle fibers of type II, which leads to increased synthesis of HSP72 proteins with a large dynamic range. Higher levels of oxidative stress in the muscles of older animals limit HSP levels and/or function in response to high-intensity contractile stress.

The level of extreme responses to physical exercise is higher after high-intensity training compared to low-intensity training. Termination of physical activity in trained individuals leads to the decrease in HSP basal expression to the baseline. Trained men demonstrate selective regulation of basal heat shock and antioxidant protein content and do not show shock response to regular running exercises.

Thermotherapy is a powerful tool for maintaining the level of sports fitness of injured athletes, people with reduced mobility and some diseases. It has been proven that to some extent, heat exposure can supplement physical activity or temporarily replace it.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ПРОТЕЇНИ ТЕПЛОВОГО ШОКУ В АДАПТАЦІЇ ДО ФІЗИЧНОГО НАВАНТАЖЕННЯ

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В оглядовій статті представлено авторську модель одного із блоків інтегрованого механізму адаптації до фізичного навантаження та супровідного помірного теплового впливу. Обговорено участь протеїнів теплового шоку в стабілізації третинної структури та у відновленні функції пошкоджених за дії температурного та фізичного стресорів протеїнів, що здійснюють регуляторну, каталітичну, транспортну, рецепторну та захисну роль, а також задіяних у процесах скорочення і розслаблення м'язів, ремоделюванні м'язової та кісткової тканини.

Ключові слова: протеїни теплового шоку (HSP), шаперони, згортання протеїнів, фізична активність.

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