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Performances in extreme environments: effects of hyper/hypobarism and hypogravity on skeletal muscle

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Abstract

Many environmental factors may affect muscle plasticity but some have exclusive characteristics that allow them to play a key role to maintain the muscle capacity to generate force; these factors are: i) the oxygen availability and ii) the load applied to muscle fibres. Hyperbarism is a condition that occurs when a man is subjected to pressure increases. To keep the lungs from collapsing, the air is supplied to him under high pressure which exposes the blood in the lungs to high alveolar gas pressures. Under this condition, the PO_2 become sufficiently increased, serious disorders may occur, such as modification of oxygen delivery and/or oxygen availability to permit regular muscle contraction. Also altitude hypobaric hypoxia induces modification of muscle capacity to generate work. Prolonged exposure to high altitude leads significant loss in body mass, thigh muscle mass, muscle fiber area and volume density of muscle mitochondria. Spaceflight results in a number of adaptations to skeletal muscle, including atrophy and early muscle fatigue. Muscle atrophy is observed in a wide range of muscles, with the most extensive loss occurring in the legs, because astronauts are no longer needed to support the body's weight. This review will describe the background on these topics suggesting the strategies to correct the specific muscle changes in presence of environmental stresses, such as the alteration in oxygen-derived signaling pathways or the metabolic consequence of microgravity that may indicate rational interventions to maintain muscle mass and function.
Key Words: Skeletal muscle, adaptations, hyperbaric, hypobaric environment, microgravity

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1. Hyperbaric environment
2. Hypobaric environment
3. Microgravity

Skeletal muscle is a highly plastic tissue that responds to changes in its pattern of activity as consequence of the mechanical and environmental stresses placed upon it. Although the signaling pathways involved in these multiple adaptations are well described, the factors and mechanisms responsible of such processes remain poorly understood [21]. Among the many environmental factors that may affect muscle plasticity, there are some having exclusive characteristics that together with the presence of motor innervation, allows them to play a key role to maintain the muscle capacity to generate force; these factors are: i) the oxygen availability and ii) the load applied to muscle fibres. Because of its high energy demand, skeletal muscle is sensitive to changes in the oxygen availability. ATP is necessary for skeletal muscle function, which is characterized by voluntary maximal or repeated force-generating capacity strictly related to both the amount and type of contractile proteins and the efficiency of the cellular mechanism of excitation-contraction coupling. However, the oxygen-depending processes, are not only correlated to the more efficiently ATP synthesis, but also to production of potentially dangerous derivatives such as reactive oxygen species (ROS). In addition, there is no doubt that microgravity derived from medium and long-term space flight has serious damaging impact upon human physiological systems. These include an extremely dangerous status as the lower limb anti-gravity muscle atrophy, which can be also evident each time that the load applied on the muscle (absence of gravity, tenotomy, bed rest, etc.) is no longer present. This review provides novel information and suggests the strategies aimed at correcting the specific changes induced in muscle, such as the alteration in oxygen-derived signaling pathways or the metabolic consequence of microgravity.

Fig. 1 *Deep dive breathing mixture of gases. Courtesy Vittorio Bianchini.*

1. Hyperbaric environment

When a man is compressed in a closed space or descends beneath the surface of the sea, he is subjected to pressure increases. To keep the lungs from collapsing, the air is supplied to him under high pressure which exposes the blood in the lungs to high alveolar gas pressures. This is known as hyperbarism. Caisson workers for example must work in pressurized areas; all divers enjoy the beneath endure increasing pressure as breath-hold and scuba (self contained underwater apparatus) divers (Fig. 1); or they work at pressure as commercial or military divers; others receive hyperbaric oxygen therapy (HBO) for the treatment of several diseases. High hydrostatic pressure can produce considerable biological effects. According to Fenn [9] enzymes, viruses, and toxins may become inactivated above 1000 atmospheres absolute pressure (ata). Pressure can affect the contractile mechanism of muscle [14], and at very high pressures muscle become rigid and stiff. The effects of pressure become much more relevant for the diver's concern when they interact with the gases in the breathing mixture. As pressure increases, the partial pressures of the gases in the breathing mix also increase proportionately according to Dalton's law. The amount of each gas dissolved in the blood and tissues depends on the partial pressure. When it becomes sufficiently increased, serious disorders may occur, such as oxygen toxicity, nitrogen narcosis, high pressure nervous syndrome (HPNS) and CO_2 poisoning [6]. Few but amazing physiological studies in humans have been performed during these years in superb hyperbaric facilities. They differentiate between the HPNS as manifested during compression and hydrostatic pressure alone. The compression syndrome as part of HPNS is characterized by changes in the EEG, the appearance of both intention and postural tremor, and neuromuscular incoordination, for example, a loss of balance. The HPNS that occurs generally at depths below 31.3 ata is consistent with findings that neuromuscular motor performance is most affected by

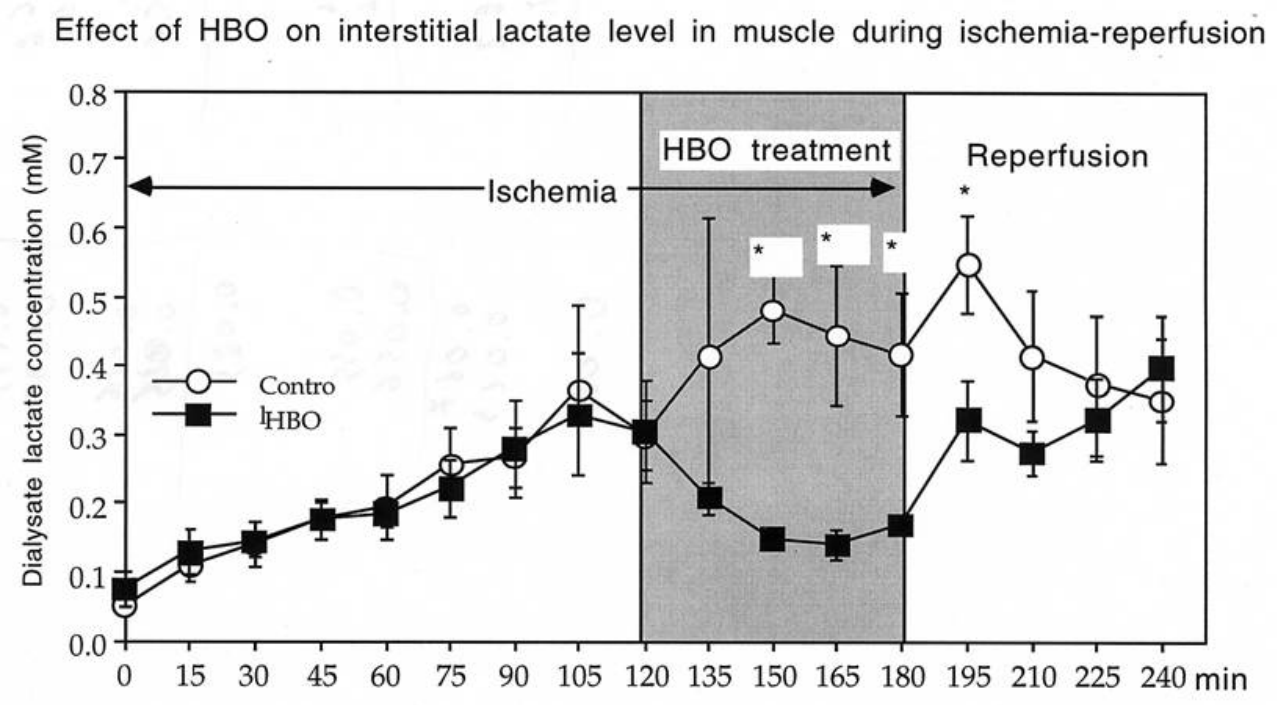

Fig. 2 *Effect of HBO on interstitial lactate level in muscle during ischemia reperfusion. From Bosco et al. [5]*

deep dives (breathing a helium-oxygen mixture) while intellectual or cognitive performance is much less affected. The opposite appears to be true in hyperbaric air where intellectual cognitive functions appear to be more degraded than pscymotor skills [28]. How much of this is a function of pressure, and how much is function of breathing helium versus breathing hyperbaric air with its aspects of nitrogen narcosis, is not entirely clear.

There are also some depth-independent effects of the immersion per se as anti-gravity vascular and respiratory pressure effects but these aspects are well detailed in reviews [6,31]. There is a direct effect on muscle contraction as well. In 1981 investigators reported the effect of hyperbaric pressure on isometric muscle contraction and work in intact animals and found the muscle tension increases as the pressure increase up to 35 ata. The rate of development of tension and total work were also increased [26]. The effects of high pressure on the mechanical response of skeletal muscle have been verified [15]. Pressure greatly increases the tension of a single isometric twitch that may reach, under high pressure conditions, the level of a tetanic response. The duration of the twitch is elongated due to an enhanced rate of rise and slowed relaxation. Ranatunga et al. [32] revealed that passive isometric tension in the relaxed state of the muscle is unaffected by increased pressure. In contrast, in the rigor state when the cross-bridges are stable, increased pressure causes an increase of isometric tension. In humans, a lack of torque reduction with higher frequency tetanic stimulation suggested that 6 ata does not impair myofilament kinetics, whereas twitch potentiation may include changes in excitation-contraction coupling [3]. Two studies of human muscle biopsy are reported in breath-hold divers. They demonstrate that prolonged habitual cold-water immersion may induce a decrease in fibre size and an increase in capillarity in female human skeletal muscle but this alteration has been decreased by prolonged habitual breath-hold diving [2,30]. Pressure has similar effects on skeletal and cardiac muscles. Both muscles exhibit a considerable positive inotropic pressure effect at steady state, most probably due to the impedance of various mechanism of Ca2+ removal from the cytosol either into cellular or extracellular compartments [12]: Increased atmospheric pressure alone is detrimental to physical performance, but this is counteracted by the elevation of inspired oxygen pressure. In most studies there were consistent reports of increased work tolerance with hyperoxia [11,24]. It has usually been assumed that the reduction of lactate level during hyperoxia is a result of alleviation of the anaerobic conditions in working muscles (Fig. 4). Another theory is that oxygen may exert its effect directly on the enzymes of the glycolytic pathways. It has been demonstrated the ammonia levels post exercise under

Fig. 3 *Himalaya Manaslu Project. Interuniversitary Institute of Myology. Coutesy from expedition team.*

HBO were much lower than the corresponding values during normobaric oxygen. Lactate levels after exercise were lower during exercise under oxygen and more pronounced with HBO. There was a fall of glucose with exercise under HBO, suggesting inhibition of glycolysis, which is a contributory factor to the rise in the level of ammonia. These studies confirm the findings of a fall of lactate and pyruvate during exercise under HBO [4]. Recently it has been demonstrated how HBO (2.8 ata) decreases lactate levels in the biceps femoris muscle of rats (. Using a microdyalis technique, authors have been able to monitor lactate as reported in Fig. 2, glucose and glycerol in real time during ischemia-reperfusion injury [35].

In the Atlantis dive series (Fig. 2) arterial blood gases were obtained at rest and during exercise at 650 m (66 ata in hyperbaric chamber) in humans breathing trimix ($He-N_2-O_2$). The investigators showed hypercapnia, respiratory acidosis, and a new finding was that at depth, arterial lactate levels were higher at rest and during exercise [5]. Recently it seems to be very intriguing the role of respiratory muscle training to avoid the work load of breathing at depth [33]. In conclusion only HBO reduces fatigue and increases physical endurance, mainly due to its effect in reducing the increase of lactic acid production and associated with physical therapy might have several advantages.

2- Hypobaric environment

Paul Bert in his most famous book “La Pression Barométrique” summarized his work on the effects of barometric pressure upon life. The fundamental physiological effect of decreasing barometric pressure is due to the concomitant fall of the O_2 partial pressure. The effects of lowering or raising the barometric pressure can be countered by increasing or decreasing the O_2 fraction in the air. Therefore, it is clear that the

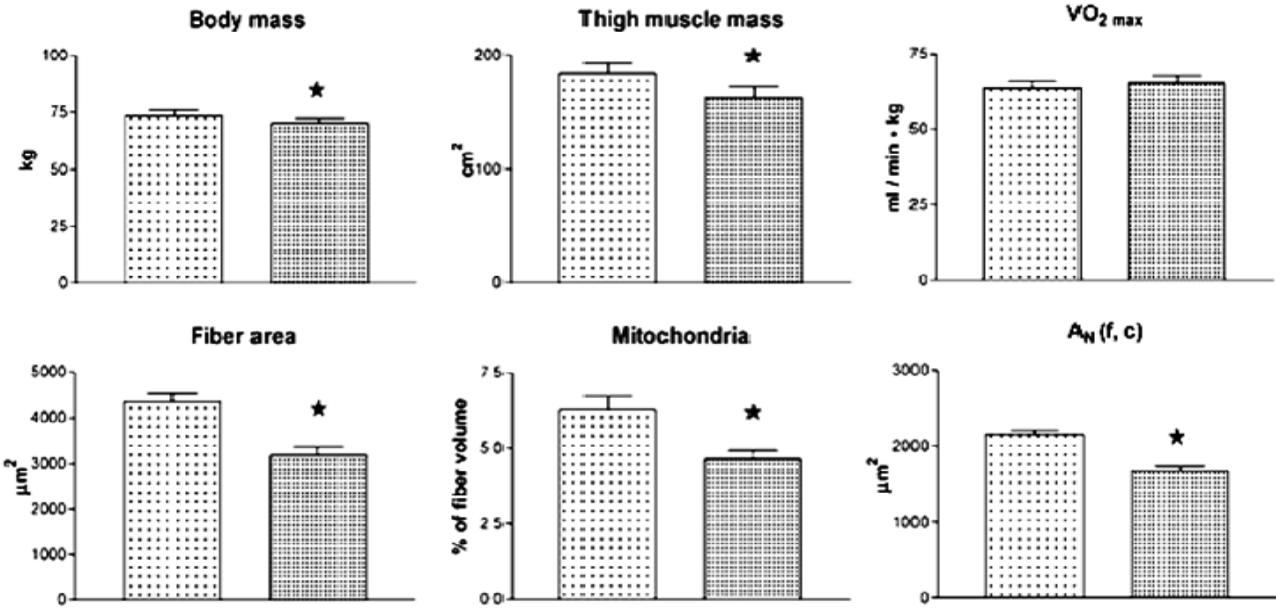

Fig. 4. *Changes in body mass, thigh muscle mass determined by computer tomography, O_2max, muscle fibre area, volume density of mitochondria, muscle fibre area perfused by one capillary [AN (f, c)], in members of an expedition to Mt. Everest. Bright columns, before departure to the Himalayas; dark columns, after returning to Switzerland from Margaria et al [24].*

main factor related to the barometric pressure change remains the hypoxia, since oxygen inhalation or recompression lead to a quick recovery [7]. Many studies analyzed the effects of short and medium time exposure to hypobaric conditions in people living at sea level while only little is known about functional adaptations to chronic hypobaric exposure of no conditioned population [8].

High altitude living produces physiological changes, including skeletal muscle capacities, as consequence of adaptation to chronic hypobaric-hypoxemic conditions (Fig. 3). Although much is known about these physiologic changes, no evident separation has been made regarding the difference between genetic and acquired adaptations.

As described twenty years ago from Cerretelli's group [17,18], a consequence of enhanced catabolism the prolonged exposure to hypoxia led to a statistically significant loss in body mass, thigh muscle mass, muscle fibre area and volume density of muscle mitochondria (Fig. 2) . The decrease in cross-sectional muscle fibre area was combined with an increase in capillary density, leading to a significant reduction of muscle fibre volume supplied by one capillary. The decrement in mitochondrial volume density was confirmed by statistically significant decreases of enzyme activities responsible for the aerobic-oxidative metabolism [10]. Since this decrease was combined with an increase of enzyme activities involved in anaerobic glycolysis, Howald and Hoppeler [16] have concluded that prolonged exposure to hypoxia together with strenuous exercise at high altitude induces an aerobic to anaerobic shift of muscle energy metabolism. Altitude hypobaric hypoxia induces modification of muscle protein synthesis and pattern as well. Repetitive muscle work in moderate quote with concomitant recovery in a well-oxygenated atmosphere elevates the structural components of local aerobic capacity by increased mitochondrial protein expression and modifications toward reduced regulation of ion gradients. Conversely, permanent hypoxia of muscle causes a net loss of mitochondria, which is compensated for by improved capillary perfusion due to a loss of contractile material. The lowering of intramuscular oxygen tension in combination with non identified contraction-related signals is the driving force for elevated angiogenic and mitochondrial protein expression with endurance training [13]. Hypobaric altitude hypoxia training can improve endurance performance. Several modalities of altitude–hypoxic training have been developed to provide the best compromise between hypoxic acclimatization and maintaining high intensity training in the face of a reduced VO_2max [36]. Prolonged exposure to high altitude leads to reduced muscle mass and performance. The fall in muscle mass follows a reduction in fiber size. Hypoxia alone does not alter capillary number and geometry in skeletal muscles of

mammals at altitude. Alterations in fiber size and aerobic enzymes depend on a number of additional factors, including activity and the level of hypoxia. With training at altitude, fiber capillary number and aerobic enzymes are increased, indicating that muscle potential for plasticity is conserved in hypoxia. Capillary number and geometry are altered in muscles of several species of birds native or exposed to higher altitude. Therefore, the capillary growth can occur in skeletal muscle in response to chronic exposure to high altitude [25]. Sustained exposure to severe hypoxia has detrimental effects on muscle structure. Short-term effects on skeletal muscle structure can readily be observed after 2 months of acute exposure of lowlanders to severe hypoxia. The full range of phenotypic malleability of muscle tissue is demonstrated in people living permanently at high altitude and the hallmark of muscle adaptation to hypoxia is a decrease in muscle oxidative capacity concomitant with a decrease in aerobic work capacity. At the molecular level, training in hypoxia results in an up regulation of the regulatory subunit of hypoxia-inducible factor-1 (HIF-1). Possibly as a consequence of this upregulation of HIF-1, the levels mRNAs for myoglobin, for vascular endothelial growth factor and for glycolytic enzymes, such as phosphofructokinase, together with mitochondrial and capillary densities, increased in a hypoxia-dependent manner [19]. Mizuno et al. investigating skeletal muscle adaptations to high altitude and a possible role of physical activity levels showed that mean fiber area was reduced in response to altitude exposure regardless of physical activity which in turn meant that with an unaltered capillary to fiber ratio there was an elevation in capillaries per unit of muscle area. Muscle enzyme activity was unaffected with altitude exposure in active and less active subjects, whereas muscle buffer capacity was increased [27].

3- Skeletal muscle adaptations to microgravity

Spaceflight results in a number of adaptations to skeletal muscle, including atrophy and early muscle fatigue. Muscle atrophy is observed in a wide range of muscles, with the most extensive loss occurring in the legs, because i) astronauts predominantly utilize their arms to effortlessly push/pull their way about the spacecraft; and ii) they are no longer needed to support the body's weight. Some studies show that a decrease up to 20% of muscle mass appeared after a relatively brief space missions (one week) [29]. For this reason the understanding of the qualitative and quantitative neuronal mechanisms which control changes in muscle function is crucial for the development of countermeasures able to contrast the loss of skeletal muscle function observed also under simulated microgravity exposure [12]. Numerous studies have examined quantitative and qualitative changes in skeletal muscle, from the whole muscle to the single myofiber from individuals undergoing real and simulated space flight for a few weeks to several months. The losses in muscle mass and function occur rapidly and can be enlarge in magnitude. Some authors report that short-duration (<15 days) spaceflights produce significant losses in muscle mass and strength (5–20%) [37]. Longer periods, more than six months in spaceflight, losses as high as 40% in muscle strength has been observed [23]. The decrease in postural muscle fiber size, diminishing of their contractile properties, slow-to-fast shift in myosin heavy chain expression pattern are known to be the main consequences of gravitational unloading. In addition analysis of microarray data revealed that more than two hundred mRNAs were significantly altered by spaceflight, the majority of which displayed similar responses to hind limb suspension, whereas reloading tended to counteract these responses. It is important to note that several mRNAs altered by spaceflight were

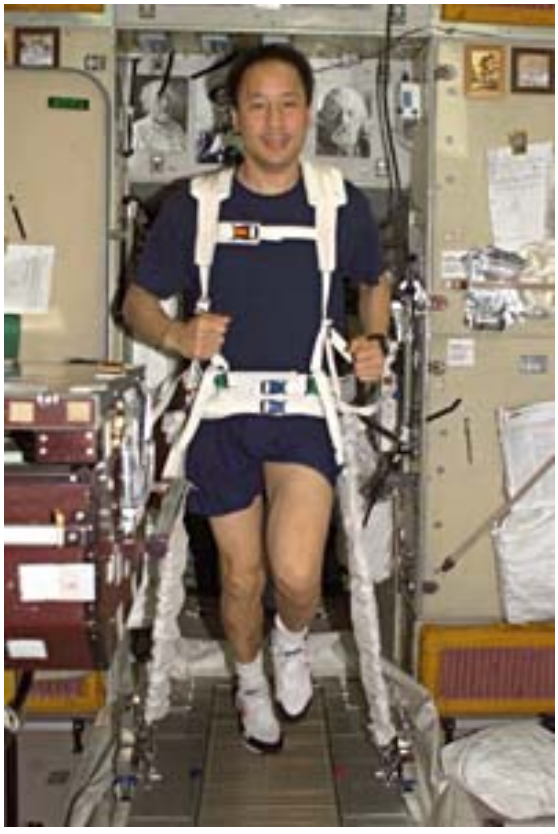

Fig. 5 *A regimen of daily exercise in space is crucial to limit muscle and bone atrophy. Astronaut Edward Lu exercising on a modified treadmill on the International Space Station. The harness and elastic cords provide resistance meant to simulate gravity (NASA: The office of Biological and Physical Research: http://microgravity.hq.nasa.gov/general_info/issphysiology_lite.html).*

associated with muscle growth [1]. In contrast, there is no change in the cell size or oxidative enzyme activity of spinal motoneurons innervating muscle fibers. Growth-related increases in the cell size of muscle fibers and their spinal motoneurons are inhibited by hind limb suspension [20]. It has been reported that the electromyogram in soleus muscle and the afferent neurogram recorded at level of the spinal cord were decreased in response to exposure to microgravity created during a parabolic flight, as well as similar phenomena were also induced by acute hind limb suspension at 1-G [22].

The Ca2+ role in skeletal muscle atrophic processes due to hypogravity was extensively studied during this last decade. The more important result found was that the resting Ca2+ level increase in skeletal muscle fibers of hind limb unloaded mice. More recent results showed that the presence of L-type Ca2+ channels' blockers prevent the Ca2+ accumulation in the fibers. Thus far, calcium-dependent signaling pathways (like calcineurin/NFAT) may play an important role in the development of some key events observed under artificial or spaceflight unloading [38].

Exercise countermeasures for long duration space crews incorporate aerobic and resistance exercise (Fig. 5). Exercise has been the primary strategy used by the space agencies in an effort to protect cardiovascular, bone, and skeletal muscle status during hypogravity exposure for extended days. The first multifaceted exercise program implemented in space was during Skylab missions in the 1970s. Using a variety of rudimentary exercise equipment, Skylab crewmembers generally maintained cardiovascular capacity while showing decrements in lower leg muscle circumference and performance [34]. Microgravity simulation studies show that resistance exercise alone can be completely efficient to prevent muscle loss, yet crews return from missions with reduced muscle mass and function. Resistance training is a form of strength training in which each effort is performed against a specific opposing force generated by resistance. Some Earth-based studies show that human skeletal muscle size and strength increases when aerobic and resistance training are performed concurrently [39]. For this reason unusual typology of physical training as 20-Hz whole body vibration training was tested to counteract the muscle mass loss induced by hypogravity. Unfortunately daily vibration stimulations, carried out on six volunteers under condition of bed rest, failed to counteract the decrease in leg muscle volume [40]. Several data derived from experiments carried out on animals and humans under condition of artificial hypogravity or during spaceflight of different duration, clearly support the idea that exercise prescription changes are necessary to protect skeletal muscle during space mission especially if it is a long-duration mission. In addition to the risk associated with reduced muscle performance, the decrease in muscle mass and the contractile phenotype shift have risk implications for sensory-motor deficits and skeletal muscle functional integrity. Applying these principles to the manned space program would lead to a more effective exercise program for skeletal muscle, reducing the risk imposed to members of crew.

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