Nonhematological Mechanisms of Improved Sea-Level Performance after Hypoxic Exposure

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ABSTRACT

GORE, C. J., S. A. CLARK, and P. U. SAUNDERS. Nonhematological Mechanisms of Improved Sea-Level Performance after Hypoxic Exposure. *Med. Sci. Sports Exerc.*, Vol. 39, No. 9, pp. 1600–1609, 2007. Altitude training has been used regularly for the past five decades by elite endurance athletes, with the goal of improving performance at sea level. The dominant paradigm is that the improved performance at sea level is due primarily to an accelerated erythropoietic response due to the reduced oxygen available at altitude, leading to an increase in red cell mass, maximal oxygen uptake, and competitive performance. Blood doping and exogenous use of erythropoietin demonstrate the unequivocal performance benefits of more red blood cells to an athlete, but it is perhaps revealing that long-term residence at high altitude does not increase hemoglobin concentration in Tibetans and Ethiopians compared with the polycythemia commonly observed in Andeans. This review also explores evidence of factors other than accelerated erythropoiesis that can contribute to improved athletic performance at sea level after living and/or training in natural or artificial hypoxia. We describe a range of studies that have demonstrated performance improvements after various forms of altitude exposures despite no increase in red cell mass. In addition, the multifactor cascade of responses induced by hypoxia includes angiogenesis, glucose transport, glycolysis, and pH regulation, each of which may partially explain improved endurance performance independent of a larger number of red blood cells. Specific beneficial nonhematological factors include improved muscle efficiency probably at a mitochondrial level, greater muscle buffering, and the ability to tolerate lactic acid production. Future research should examine both hematological and nonhematological mechanisms of adaptation to hypoxia that might enhance the performance of elite athletes at sea level. **Key Words:** ERYTHROPOIESIS, EFFICIENCY, MUSCLE BUFFERING, MUSCLE PH

Endurance athletes have been using altitude training for nearly half a century in pursuit of improving sea-level performance (47). The effect of altitude training on endurance performance has been researched extensively, and there is a widespread acceptance that altitude training can enhance sea-level endurance performance (70), although the scientific evidence is controversial and tends to indicate no significant benefit (66). However, because the relative improvement in performance required by a top individual athlete to increase their chance of winning medals at international competition is about 0.5% (32), it is not surprising that with sample sizes typically less than 20, many studies have been underpowered to detect a change of this magnitude using conventional statistics. For the purposes of this review, altitude is defined as follows; sea level = 0–1000 m, low altitude = 1000–2000 m, moderate altitude = 2000–3000 m, high altitude = 3000–5000 m, and extreme altitude = 5000–8848 m. The traditional approach to altitude training involves athletes living and training at low to moderate (1500–3000 m) natural altitude. Because training quality can suffer by training at moderate to high altitude, a recent approach has been for athletes to live/sleep at altitude and train near sea level, the so-called live high–train low (LHTL) method (46). Because the geography of many countries does not readily permit LHTL a further refinement involves athletes living at simulated altitude under normobaric conditions and training at, or close to, sea level (65). In recent years, endurance athletes have used several new devices and modalities to complement the LHTL approach. These modalities include normobaric hypoxia via nitrogen enrichment generated with molecular sieves that allow athletes to undertake LHTL; as well as supplemental oxygen to simulate normoxic or hyperoxic conditions during exercise/sleep at natural altitude. Intermittent hypoxic exposure is another method involving brief periods (minutes to a few hours) of high or extreme hypoxic exposure to stimulate erythropoietin (EPO) production, although data to support any performance benefits for athletes competing at sea level are minimal and inconclusive (38).

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It has been suggested that the main mechanism for improved sea-level performance after altitude exposure is an increase in erythrocyte volume and red cell mass, leading to an increase in maximal oxygen consumption ($\dot{V}O_{2max}$), and that there are no identifiable alternatives that can increase performance after altitude acclimatization (47). Among studies with control groups there are a number of reports that the so-called LHTL protocol improves sea-level performance by about 1.0–1.5% in events lasting 45 s to 17 min. We have published a summary of our findings from several studies of simulated altitude (28) but others report similar findings (46), including with nationally-competitive level athletes (74). Although some authors have explicitly related the change in sea-level performance to the change in serum EPO (46,47), the correlation for the change in $\dot{V}O_{2max}$ versus the change in red blood cell volume yielded an $r^2 = 0.137$ (46). This means that 86% of the variance in $\dot{V}O_{2max}$ could be attributed to factors other than the change in red blood cell volume, and it is important to be cognizant that $\dot{V}O_{2max}$ is not the sole determinant of performance (18), particularly among elite athletes. Given adequate exposure to altitude (high enough, long enough, and for enough hours per day), there will be an increase in serum EPO concentration that may lead to an increase in erythrocyte volume and red cell mass, $\dot{V}O_{2max}$ and possibly performance. However, there is substantial evidence from numerous studies that improvements in endurance performance at sea level can be obtained through altitude exposure via nonhematological mechanisms, which will be the major focus of this review. Where possible, this review will concentrate on data obtained from athletes in order to promote relevance to them, but where appropriate experiments have not yet been conducted we have relied upon data from a diverse range of contexts.

THE ERYTHROPOIETIC PARADIGM

The prevailing paradigm of adaptation to hypoxia is that the lower partial pressure of oxygen associated with altitude induces EPO production in the kidneys, which in turn stimulates the production of red blood cells in the bone marrow, an increase in $\dot{V}O_{2max}$ and thus performance (46,47,74). If the dose of altitude is sufficient and exposure is of adequate duration there is no question about the first step in this cascade (45), albeit that EPO also lowers plasma volume by reducing the activity of renin–angiotensin–aldosterone axis (51). The threshold altitude for a sustained increase in blood EPO concentration is about 2200 m (81), which is a consequence of the sigmoidal shape of the oxyhemoglobin dissociation curve. During exposure to continuous terrestrial altitude, serum EPO reaches a peak within 24–48 h and thereafter declines to be near baseline levels (~10 IU·L^{-1}) after approximately 1 wk (27) (Fig. 1). The half-life of serum EPO has been estimated at 5.5 h and during LHTL exposure EPO may not be elevated for much of the day, giving no stimulus for accelerated erythrocyte

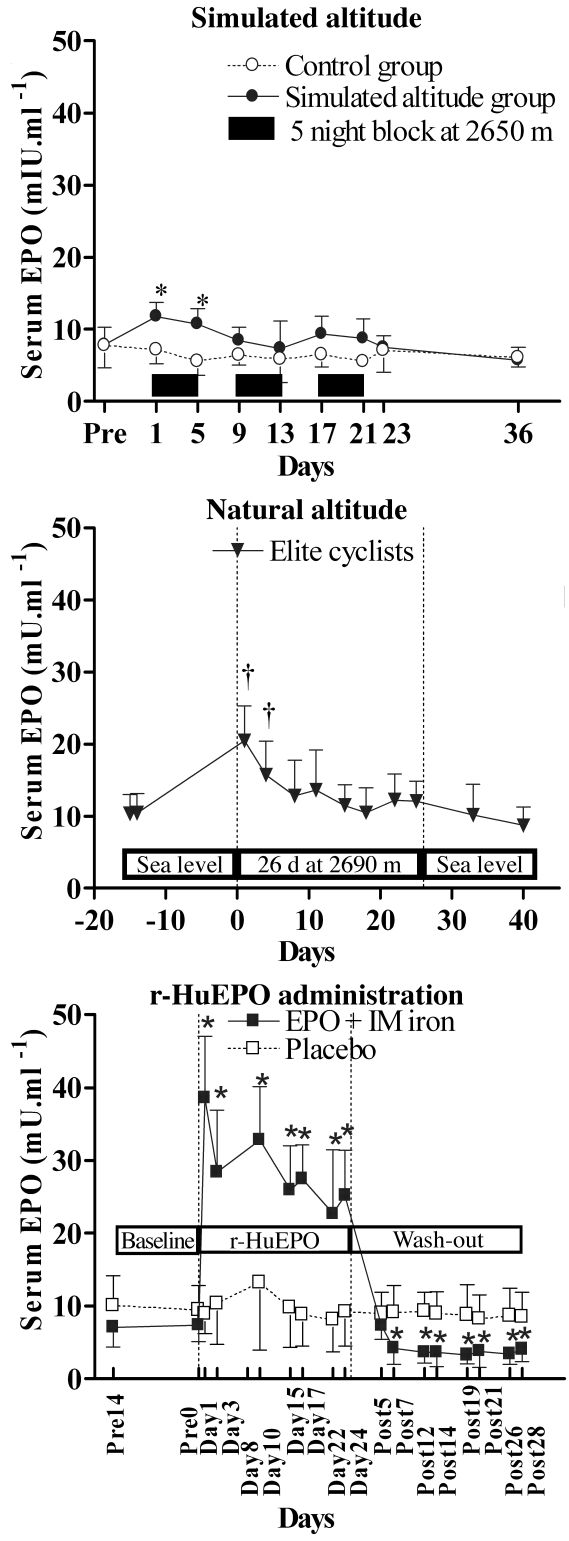

FIGURE 1—Serum erythropoietin (EPO) response of (*a*) elite middle-distance runners to sleeping in simulated altitude (2650 m) for three blocks of five nights, separated by three nights spent at 600 m, Canberra, Australia; (*b*) elite track cyclists ($N = 11$) to a 26-d altitude training camp in Toluca, Mexico, during February 2000; (*c*) recreational athletes injected with recombinant human erythropoietin (r-HuEPO) for 25 d, 3 d·wk^{-1}, at 50 IU·kg^{-1} and with supplemental intramuscular (IM) iron. Values are means and standard deviations. Statistically significant differences ($P < 0.05$): † difference versus prealtitude value; * difference between groups. From Hahn and Gore (27); used with permission.

production (28). Grover and Bärtsch suggest that the initial increase in serum EPO represents the period when EPO production exceeds its consumption by the bone marrow, which is followed by a fall when accelerated erythropoiesis leads to greater EPO consumption, and finally a new equilibrium with higher EPO turnover that is not detectable via measures of serum EPO (26). The precise metabolic fate of serum EPO remains obscure but is likely via EPO-receptor mediated uptake mostly in the bone marrow (34). Circumventing the limitations of serum EPO by focusing on the next step in the erythropoietic cascade after hypoxic exposure, we have often been unable to detect an increase in young red blood cells (reticulocytes) when using flow cytometry (3–5,23), which is considered far superior to manual methods of counting reticulocytes. In each of these studies (3–5,23), iron status was measured in all subjects and iron supplements were taken to promote red cell production. We have also taken care to use appropriate control groups, because training alone can stimulate reticulocytes, as discussed by Ashenden et al. (3). Even with 23 nights at a 3000-m simulated altitude (4) and 26 consecutive days in Mexico at 2690 m (27), we did not observe an increase in reticulocytes. Nevertheless, in the latter study there was a modest (13%) increase in soluble transferrin receptor (sTfr) by day 11, which is considered a prime indicator of the erythroid mass as well as an indicator of infection and iron status. However, strenuous exercise alone can increase sTfr, at least in rats (61). We cannot discount the possibility that apart from the Mexico study (27), the dose of hypoxia may have been insufficient (<12 $h \cdot d^{-1}$ for < 4 wk) (64) in our previous studies (3–5,22,70) to induce a substantial increase in red blood cell production.

Unlike the response to hypoxia, we have been able to measure a mean 12% increase in hemoglobin mass and an associated 7% increase in $\dot{V}O_{2max}$ when using recombinant human erythropoietin (r-HuEPO) (59). Recreationally active subjects were injected subcutaneously with 50 $IU \cdot kg^{-1}$ r-HuEPO three times per week for at least 3 wk, to develop a blood-based method to detect r-HuEPO for the International Olympic Committee. The serum EPO concentrations at 24 h after injection were 3–3.5 times greater than baseline levels (~10 $IU \cdot L^{-1}$) (59), indicating that peak values were likely double this. In an athletic cohort, it has been reported that the half-life of elimination is 35.5 h (77) when r-HuEPO (50 $IU \cdot kg^{-1}$) is administered subcutaneously on a daily basis. In the latter study, serum EPO concentration was generally above 30 $IU \cdot L^{-1}$ and, during each 24-h period, averaged 40 $IU \cdot L^{-1}$. These values for serum EPO concentration after r-HuEPO are twice the *peak* values we measured at the 2690-m (27) terrestrial altitude and are much more sustained. Interestingly, for end-stage renal failure patients, it has been recommended that for effective therapy serum EPO should not fall below a value 30–100 $IU \cdot L^{-1}$ above basal levels (14). It is clear that with a sustained terrestrial altitude of 2690 m and with a simulated altitude of a similar level, there is an approximate doubling of serum EPO concentration for a few days, but there is a much smaller and less sustained EPO response than that achieved by repeated r-HuEPO injections (Fig. 1) (27).

The performance benefits of blood doping (73) and by inference use of r-HuEPO are irrefutable, but after a decade of experiments both with simulated and terrestrial altitude, we are unconvinced that the evidence for accelerated erythropoiesis is compelling for highly trained *athletes*, undertaking *2–4 wk* sojourns, to a *moderate* altitude (27,47). In the last few years, we have established unequivocally via double-blind experiments that intermittent severe hypoxia of 5 min at about 5000 m and 5 min at normoxia, 1 $h \cdot d^{-1}$ for 4 wk (38) and 3 $h \cdot d^{-1}$ at 4000–5500 m for 20 consecutive days (23) does not increase the number of red blood cells in athletes, and the latter despite large increases in endogenous EPO. Consequently, there is not an obligatory increase in the total hemoglobin mass of an athlete after a transient increase in serum EPO. Moreover, despite the limitations of cross-population comparisons, an increase in the number of red blood cells in response to chronic hypoxia (living at moderate to high altitude) is not a universal phenomenon. The pioneering work in the 1950s about hypoxic adaptation was conducted on Andean subjects (62) and their physiological responses of high hemoglobin concentration, decreased ventilatory drive, and increased pulmonary artery pressure, may not be the most beneficial responses for living at high altitude (78). However, the response of an individual athlete to hypoxia is more relevant than a population response, and a highly variable response of athletes to hypoxia has been noted (15). Furthermore, it is not yet established whether an individual athlete will respond in a similar manner to repeated bouts of the same altitude months or years apart.

CROSS-COMPARISONS OF THE ERYTHROPOIETIC PARADIGM

The predominance of studies investigating adaptation to high altitude has focused on the blood because of its role in oxygen transport and the inference that the universal human adaptive response is a proliferation of red blood cells, because it has been exhibited by Europeans with brief exposure to altitude and by Andean high-altitude natives with millennia of exposure (9). Natives to the Andean highlands have been characterized as having a high hemoglobin concentration [Hb] relative to their sea-level counterparts (1). In contrast studies of blood samples from Sherpa and Tibetan males residing at 3600–4000 m have mean [Hb] 1–2 $g \cdot dL^{-1}$ lower than predicted on the basis of Andean data (11). Beall et al. (9) compared the mean [Hb] in Tibetan natives of the Himalayas (3800–4065 m) with lifelong Bolivian highlanders of the Andes (3900–4000 m), and both groups of altitude natives were compared with sea-level residents from the third National Health and Nutrition Examination Survey (NHANES III) conducted in the

United States. The Tibetans had a significantly lower [Hb] than the Bolivians, and the Tibetans also had [Hb] closely resembling that of the sea-level residents from NHANES III. The contrasting [Hb] in these two populations, which both have millennia of exposure to the same high-altitude stress, indicate that the human body is capable of more than one successful response to hypoxia (9). The highlanders from the Andes seem to have a hematological response to living at altitude with higher [Hb] (8), whereas the Tibetan adaptations are nonhematological and include having a 1.5 times higher resting ventilation and double the hypoxic ventilatory response (HVR) of the Bolivians (12). It has been suggested that the Tibetan highlanders are better equipped to cope with hypoxic conditions compared with the Andean natives (8) because individuals with high hematocrit values (50–55%) are at increased risk of problems associated with high blood viscosity and are more susceptible to chronic mountain sickness (84). The increased incidence of this sickness in the Andeans compared with the Tibetans indicates that the high [Hb] in the Andeans may lead to a loss of adaptation and pathological decreases in ventilation, HVR and blood oxygen saturation (8). Moore et al. (57) have suggested that Tibetans are better adapted to live at high altitudes compared with Andeans because of enhanced oxygen transport, leading to exceptional exercise performance at altitude. The superior performance capacity at altitude of the Tibetan Sherpas is not attributable to an exceptional $\dot{V}O_{2max}$ but, rather, better exercise economy, better lung function, higher maximal cardiac output, and better levels of blood oxygen saturation (84). Furthermore, it has recently been reported that high-altitude natives of Ethiopia demonstrate a similar [Hb], serum EPO concentration, and oxygen saturation within the normal sea-level range (10). It seems that Ethiopian highlanders, like Tibetans, have exceptional adaptations of oxygen uptake or delivery that are not associated with an increased red blood cell production in the presence of a hypoxemic stimulus (10). The success of Ethiopian distance runners, who predominantly live in the highlands of Ethiopia, demonstrates the performance capabilities of these people, which is apparently not attributable to their increased red cell volume or superior $\dot{V}O_{2max}$ as a result of residing at altitude. Notwithstanding the inherent limitations of cross-population comparisons, these studies demonstrate that the EPO paradigm is not the sole mechanism of chronic adaptation to high altitude.

MOLECULAR RESPONSES TO HYPOXIA: HIF-1 TARGETS

In the last decade researchers have begun to probe the fundamental responses to hypoxia at the level of gene expression (85). It has been shown that a transcription factor called hypoxia inducible factor-1 (HIF-1), which is present in every tissue of the body, is the global regulator of oxygen homeostasis and plays a critical role in the cardiovascular and respiratory responses to hypoxia (72). HIF-1 expression is tightly regulated by oxygen tension, with HIF-1 virtually undetectable under normoxia due to rapid degradation of the HIF-1 sub-units thorough the ubiqiquitin-proteasome pathway (40). In normoxic conditions the half-life of HIF-1 is about 5 min, but when exposed to hypoxia its half-life is increased by about 30 min, allowing HIF-1 to stabilize and accumulate in the cells, leading to transcription of specific genes. HIF-1 expression and protein levels rapidly decay when cells are returned to normoxia (33). HIF-1 was identified for its role in regulating the transcription of the EPO gene (80); however, HIF-1 is also induced by hypoxia in many cell lines and activates multiple genes, which in turn encode proteins that mediate adaptive responses, other than hematological ones (68). Parameters activated by HIF-1 include EPO and transferrin for iron metabolism and red cell production; vascular endothelial growth factor (VEGF) and others for angiogenesis/cell survival; glycolytic enzymes including phosphofructokinase (PFK), hexokinase and lactate dehydrogenase that are important for energy metabolism; glucose transporters 1 and 3, and monocarboxylate transporters 1 and 4 critical for glucose uptake and lactate metabolism by the muscles; carbonic anhydrase for pH regulation; nitric oxide synthase and heme oxygenase, which produce the vasodilators nitric oxide (NO) and carbon monoxide; and tyrosine hydroxylase that codes for a pivotal enzyme for dopamine synthesis and accelerates ventilation (68). The plethora of HIF-1 mediated responses to hypoxia implies that an increase in EPO concentration could be concurrent with other physiological changes such as increased carbohydrate metabolism, increased ventilation, enhanced muscle buffering, and more efficient use of oxygen in the muscles. Thus, the responders and nonresponders identified by Levine and Stray-Gundersen (46) might have their 1.5% performance improvement in 5000 m run time caused by some or all of the factors listed. It has been reported that VEGF is increased in swimmers undergoing training at the modest altitude of 1886 m (2) and others have confirmed that VEGF mRNA is upregulated when untrained subjects commence intense training in hypoxia equivalent to 3850 m, with no equivalent increase in VEGF mRNA apparent with the same training in normoxia (79). However, one should be cautious interpreting elevated levels of any mRNA because an increase does not necessarily indicate a greater quantity of the relevant protein (24), let alone the downstream effects such as greater capillarization secondary to an increase in VEGF. Nevertheless, recently it has been shown that untrained subjects exercising intensely in hypoxia increase the expression of mRNA for PFK (79) contrary to our previous review of the literature which suggests that glycolytic enzymes activities are reduced in athletes training at moderate altitude (22). Our own work with LHTL has demonstrated a decreased rate of appearance of lactate

during exercise at 85% $\dot{V}O_{2max}$ (16) and reduced muscle Na^+/K^+ ATPase activity (6). We have recently reported an enhanced hypoxic ventilatory response in athletes after LHTL (76), and have previously observed an increase in submaximal ventilation after exposure to LHTL (22). Overall, there is compelling evidence at both the molecular and whole body level that the response to hypoxia is multifaceted and that merely attributing any small performance benefits to increased numbers of red blood cells may be too simplistic.

IMPROVED ECONOMY OF EXERCISE: INCLUDING MITOCHONDRIAL EFFICIENCY

More use of oxygen is a teleologically appealing adaptation to a shortage of oxygen in tissues when exposed to a hypoxic environment. Indeed, studies from seven independent research groups have demonstrated 3–10% improvements in exercise economy, as demonstrated by reduced oxygen consumption during submaximal exercise, after various forms of altitude acclimatization (22,25,31,41, 42,52,58,70,71) (Fig. 2). With the association between economy and performance well documented (69), the ability to improve exercise economy after altitude training is likely worthwhile. On the other hand, the improvement in economy after altitude exposure is strongly contested by other researchers (50). Interestingly, the recent review by Lundby et al. (50) reports that there were no significant changes in economy of lowlanders after 8 wk of exposure to 4100 m, despite there being an approximately 15% reduction in submaximal VO_2 compared with the significant 3–10% changes listed above. Similarly, altitude natives had a 15% lower submaximal VO_2 than sea-level residents (Fig. 3), consistent with the observations of others (31,52).

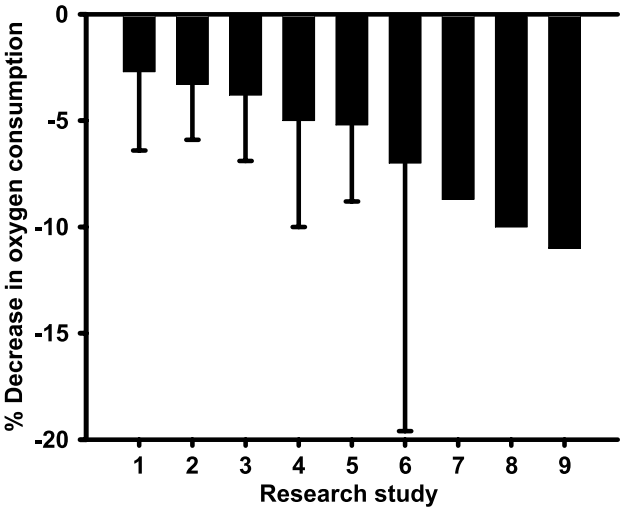

FIGURE 2—Percent decrease in oxygen consumption during submaximal exercise after various forms of altitude acclimatization, demonstrated in nine research studies from seven independent research groups. Values are mean ± SD (SD present for six of nine studies). 1) Three hours per day for 2 wk of intermittent exposure to normobaric hypoxia (12.3% FIO_2) (42). 2) Twenty nights of about 8 h per night of sleep at a simulated altitude between 2000 and 3100 m, and training near sea level (600 m) (70). 3) Twenty-three nights of about 10 h per night of exposure to a 3000-m simulated altitude (22). 4) Twenty-nine days of about 12 $h \cdot d^{-1}$ of exposure to simulated LHTL at 3000 m (58)). 6) Exposure to intermittent hypobaria of 4500 m, 3× 90 $min \cdot wk^{-1}$, for 3 wk (41). 7) Five to six nights at 2500 m followed by 8 to 12 nights at 3000–3500 m, sleeping at simulated altitude and all training at 1200 m (71). 7) Mountain climbing for 3 wk to 6194 m (25). 8 and 9) Long-term residence at 3500–4500 m (31,52).

Plausible mechanisms for improvement in exercise economy after a period of altitude exposure include a decreased cost of ventilation, greater CHO use for oxidative phosphorylation, and/or an increased ability of the excitation and contraction process to perform work at lower energy costs (25). Work from our research group suggest that the physiological mechanisms eliciting an improved economy after hypoxic exposure appear unrelated to decreased ventilation or a substantial shift in substrate use (70). Therefore, it is possible that the main mechanisms responsible for improved economy at sea level after a period of altitude exposure are either an increase in the ATP production per mole of oxygen used (31) and/or a decrease in the ATP cost of muscle contraction (60). A reduced energy requirement of one or more processes involved in excitation and contraction of the working muscles has been previously postulated by Green et al. (25), possibly as a result of a reduction in by-product accumulation, such as ADP, inorganic phosphate and H^+, that occurs after altitude acclimatization, which increases the amount of free energy released from ATP hydrolysis and depresses the need to maintain hydrolysis rates at preacclimatized levels.

The concept of improved economy of exercise after hypoxic exposure being attributable to changes in the coupling of ATP demand and supply in the working muscles was first postulated by Hochachka (30). Performance in endurance events is defined as $\dot{V}O_{2max} \times \dot{V}O_{2fracmax} \times$ efficiency, where $\dot{V}O_{2fracmax}$ is the fractional use of $\dot{V}O_{2max}$ that an athlete can attain during an event (18). Efficiency of physical work is an overall measure of how effective the body is at converting substrates into external work in the form of ATP (56). Explaining how economy might improve after altitude exposure requires an understanding of energy metabolism via oxidative phosphorylation, to determine the processes that may be altered after exposure to hypoxia and ameliorate energetic efficiency. The high ATP use required for endurance exercise can only be supported by mitochondrial oxidative phosphorylation, and, consequently, mitochondrial efficiency has a direct influence on whole-body efficiency (60).

At the cellular level, mitochondrial efficiency defines the coupling between ATP formation and substrate oxidation and is based primarily on intramuscular oxidation of NADH by molecular oxygen with some reducing equivalents also provided by reduced flavins ($FADH_2$). The energy-rich intermediate of oxidative phosphorylation is a proton gradient across the mitochondrial membrane. However, dissipation of the proton gradient by leakage of H^+ over

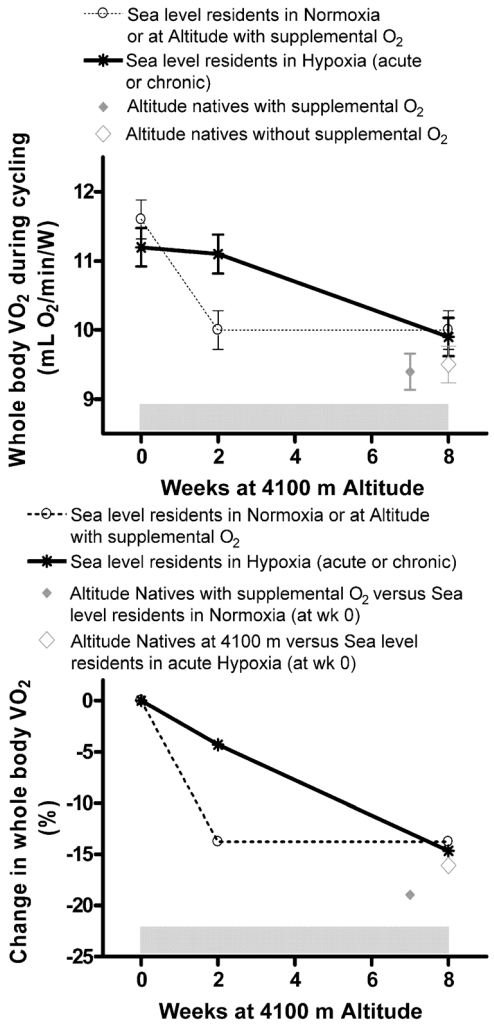

FIGURE 3—Absolute (*top panel*) and relative changes (*bottom panel*) in submaximal oxygen consumption from data reported by Lundby et al. (50). The tests on sea-level residents (N = 8) at week 0 were conducted at sea level in normoxia and 1 wk later at sea level in acute hypoxia equivalent to 4100 m. The tests at weeks 2 and 8 were conducted after 2 and 8 wk, respectively, living at 4100 m with supplemental O_2 (to generate a sea-level-equivalent atmosphere) and in the ambient hypoxic conditions. The altitude residents (N = 7) were tested at a natural altitude of 4100 m, and the time between tests was about 1.5 h. Values are means and SD where error bars are included.

the mitochondrial membrane can uncouple ATP formation from substrate oxidation and decrease mitochondrial efficiency (39). A well known example of uncoupling is the mitochondrial uncoupling protein (UCP) of brown adipocytes (UCP1), which dissipates energy of substrate oxidation as heat (63). The uncoupling protein UCP3 is present in human skeletal muscle, although its function is unclear. However, muscle UCP3 content is reduced with endurance training and a decrease in UCP3 has recently been associated with better gross efficiency in cycling (56). It has also been demonstrated that ATP synthesis in skeletal muscle is increased in UCP3 knockout mice (17), consistent with its role in mitochondrial efficiency. A change in muscle UCP3 protein content after hypoxic exposure may help explain improved mitochondrial efficiency and is an area that deserves further investigation. Another mechanism for improved mitochondrial efficiency is production of NO attributable to hypoxic exposure. As mentioned previously, one of the targets of HIF-1 accumulation is production of NO via inducible NO synthase. NO has been reported to inhibit the terminal enzyme (cytochrome *c* oxidase) in the mitochondrial electron transport chain, in competition for oxygen in a reversible manner. This response has the ability to increase oxygen delivery to tissues and maintain ATP levels in conditions of reduced oxygen availability (53).

It has also been established that low sensitivity to cytosolic ADP and the control of respiration by the creatine kinase (CK) system, is a hallmark of fatigue resistant oxidative muscles. At the local level, mitochondrial respiration is driven by the ratio of creatine (Cr) to creatine phosphate instead of cytosolic ADP, with mitochondrial CK being coupled to ADP production and translocation. Better coupling of energy production to energy utilization by the CK system may increase mitochondrial efficiency, by delaying anaerobic energy production because local ATP and ADP levels remain in relative homeostasis for longer (60). Another way of improving mitochondrial efficiency is by delivering the reducing equivalents of substrates into the mitochondrial respiratory chain at different steps. Oxidation of cytosolic NADH involves import of reducing equivalents into the mitochondria by the malate–aspartate shuttle or the glycerophosphate shuttle. The glycerophosphate shuttle omits the first phosphorylation step at complex I, yielding a lower amount of inorganic phosphate incorporated into ATP per mole of oxygen consumed (decreased efficiency), and accompanied by faster rates of respiration and increased thermogenesis (39). On the other hand, greater use of the malate-aspartate shuttle to oxidize NADH would produce more ATP per mole of oxygen used, leading to better muscle efficiency. In an animal model, it has been reported that 25–30 d of simulated altitude exposure equivalent to 5000 m via hypobaria for 5–6 $h \cdot d^{-1}$ resulted in improved mitochondrial efficiency (49). Lukyanova claims that the mechanisms responsible for this improved efficiency are related to the ability to maintain high coupling of oxidative phosphorylation and synthesis achieved by an increase in number of mitochondria in the cells accompanied by a reduced cytochrome content possessing a higher activity, new kinetic properties of NADH oxidation allowing maintenance of key enzyme activity in conditions of high NADH reduction under hypoxia, and reducing the role of the less efficient succinate oxidase pathway. In humans, 6 wk of simulated hypoxic training at 3000 m twice a week for 20 min resulted in adaptations of the athlete's skeletal muscle allowing better coupling between the energy use and production sites to promote more efficient oxidative pathways (60). Specifically, mitochondrial sensitivity to ADP was depressed by 57% in the group exposed to hypoxia compared with the control group, represented by a higher half-maximal velocity (K_m) for ADP. After hypoxic training, the

$K_m/(K_m + Cr)$ ratio increased twice as much (124 vs 66%) in the hypoxic training group compared with the control group. This ratio reflects improved efficiency of mitochondrial CK and oxidative phosphorylation coupling and, taken together with reduced ADP sensitivity, indicates more efficient energy metabolism in the mitochondria (60).

MUSCLE PH REGULATION AND BUFFER CAPACITY (βM)

At rest, muscle pH is regulated by the Na^+/H^+ exchanger that is activated strongly when internal pH is reduced, thereby functioning as a safety system against major changes in internal pH (35). During intense exercise, when lactate production is high, the lactate/H^+ transport system handles most of the H^+ produced. The transport of lactate in skeletal muscle is facilitated by two known monocarboxylate transporters, MCT1 and MCT4, and the transport of lactate is coupled to the transport of H^+ in a 1:1 ratio (36). Therefore, an increase in MCT1 and MCT4 protein expression could minimize perturbations in intracellular pH, and training studies have reported increases in the density of these membrane transporter proteins (19).

There have been relatively few studies that have investigated changes in membrane transport proteins in response to altitude exposure. It has been demonstrated in rats that hypoxia stimulates glucose transport by translocation of Glut-4 from the intracellular compartments to the plasma membrane of skeletal muscle. It has also been shown that the major signaling agents for the hypoxia-mediated glucose transport in skeletal muscle is dependent on the presence of AMP-activated protein kinase and may also be effected by reactive oxygen species (43). Clark et al. (16) measured MCT1 and MCT4 protein content in skeletal muscle of well-trained individuals after 20 nights of simulated moderate LHTL. Although this study reported a decrease in lactate rate of appearance, there was no change in MCT1 and MCT4 protein abundance. Because the lactate/H^+ transport system is thought to be more active during exercise than at rest (37), it may be that only sleeping under hypoxic conditions is not an adequate stimulus to increase MCT1 and MCT4 protein expression. On the other hand, Juel et al. (37) reported a fivefold increase in MCT1 proteins in the erythrocytes after 8 wk of acclimatization to 4100 m in untrained humans. The authors of this study suggest that if the increase in the MCT1 in erythrocytes is functional then it could be expected to enhance the lactate/H^+ transport across the erythrocyte membrane and act as an important dilution space during intense exercise when there are fast changes in plasma lactate and pH (37). Interestingly, it was reported that there was no change in the skeletal muscle MCT1 and MCT4 transporters (37). These results are similar to those of Clark et al. (16), and in both studies the subjects performed no vigorous exercise under hypoxia. In contrast, Zoll et al. (86) reported an increase in the muscle mRNA concentration of MCT1 (44%) in nine well-trained runners after 6 wk of training (12× 24- to 40-min sessions) under hypoxic conditions (3000-m simulated altitude) compared with a control group. Run time to exhaustion at $\dot{V}O_{2max}$ increased in the hypoxic trained group with no change in the maximal lactate accumulation. The authors concluded that the increase in MCT1 mRNA allowed an improvement of lactate exchange and removal that may lead to the slower decline in pH at a given running velocity, thereby allowing the athletes to run longer (86). The capacities of the pH-regulating transport systems are not only dependent on the protein density and activation of the individual transport proteins but also on the carbonic anhydrases (CA) (37). The reactions catalyzed by CA function as H^+ acceptors or donors and thereby influence the rate of H^+ and HCO_3^- transport (21). An increase in CA IV isoforms in glycolytic muscle fibers was reported after 8 wk of acclimation to altitude (37). Furthermore, it has been demonstrated that the muscle mRNA of CA IV has been increased after 6 wk of training in hypoxic conditions (86). Collectively, these few studies indicate that altitude acclimatization may alter the transport systems that are involved with changes in dynamic buffering capacity.

In addition to transport of lactate and H^+, the ability of skeletal muscle to buffer H^+ is important for pH regulation and changes in acid–base status have been proposed as a potential mechanism for improved performance after altitude exposure (75). A very rapid physiological response to hypoxia is hyperventilation to raise the alveolar PO_2, which leads to respiratory alkalosis and a decrease in PCO_2 resulting in a decreased H^+ level and a corresponding increase in pH (29). In turn there is increased renal bicarbonate excretion, which is the primary buffer of lactic acid (13). The elevated muscle lactate concentration and $[H^+]$ in addition to a compensatory respiratory alkalosis during the early stages of altitude acclimatization results in a greater decrease in pH per millimole H^+ release (82). This may explain why training at altitude has been shown to increase muscle buffering (βm) and attenuate the degree of acidosis (55,67). Mizuno et al. (55) reported that training at 2700 m for 2 wk increased βm by 6% in well-trained runners. Furthermore, a significant correlation was observed between the relative increase in βm of the gastrocnemius and short-term (4–6 min) running time ($r = 0.83$, $P < 0.05$). Unfortunately, in that study (55), there was no control group, so it is difficult to assess the effect of hypoxia independent from the training load that was performed during the investigation. Saltin et al. (67) report that an increase in βm of the gastrocnemius in six runners after 2 wk training at about 2000 m compared with the control group that trained at sea-level. A decrease in βm was observed when a group of high-altitude natives trained in normoxia (20). Gore et al. (22) were able to demonstrate that sleeping at altitude and training at sea level (23 nights of sleeping at simulated altitude of 3000 m) increased βm by about 18% in the vastus lateralis. However, these findings were not reproduced in our subsequent study (16). Differences in

results may be attributable to the 13% higher simulated altitude in the first study (22).

POTENTIAL DETRIMENTAL EFFECTS OF HYPOXIA

A balanced review of the effects of hypoxia on sea-level performance is incomplete without some consideration of its potentially unfavorable effects, which include hypoxia's impact on cardiac function, anaerobic metabolism, muscle function, sleep and immune function. At high altitude, there is a depression of cardiac output caused mainly by a reduction in stroke volume and possibly lower myocardial contractility (27) and even after more moderate altitude (4 wk of LHTL at 2500 m) there was a trend toward a lower cardiac output and a significantly larger arteriovenous difference during treadmill running at race pace at sea level (46). On the other hand, at a low altitude (1980 m), LHTL for 2 wk was associated with improved left ventricular contractility (48).

Although controversial (83) several months spent at extreme to high altitude may be associated with a reduction in anaerobic metabolism, as evidenced by reduced lactate concentrations during exercise compared with levels attained upon recent ascent to altitude. However, at altitudes and durations more typical of those used by athletes (LHTL at 3000 m for 23 nights) the evidence from both muscle and blood data did not indicate that lactate accumulation during intense exercise was depressed, nor was the calculated anaerobic ATP production (22). High altitude suppresses muscle Na^+/K^+-ATPase content and activity and this enzyme is critical to maintaining membrane excitability and hence is linked to fatigue (6). However, exposure to moderate altitude (3000 m LHTL for 23 nights) led to a small (3%) decrease in Na^+/K^+-ATPase activity and no change in plasma K^+ regulation or work output during high intensity cycling (6). This suggests that this duration of LHTL was insufficient to adversely affect muscle function, but does not preclude more deleterious effects with higher or longer duration exposures.

Sleep is essential for athlete recovery, but even at moderate altitudes (2650 m) there is sleep disturbance and even periodic breathing among some susceptible athletes (44). A further consideration for athletes that have to travel abroad to access suitable altitudes is the increased propensity to illness (7) as evidenced by a suppressed immune function (54). Overall, the negative effects of hypoxia seem more pronounced at high to extreme altitude, albeit that sleep and immune function of some athletes are likely compromised even at moderate altitude.

CONCLUSION

In summary, this brief review has detailed the large body of literature that demonstrates improvements in sea-level performance after a period of altitude exposure/training may have a multifactorial etiology and are not solely dependent on increasing red cell volume via erythropoiesis. Mechanisms responsible for this observed improvement in performance after exposure to hypoxia appear to be a HIF-1 driven response at a molecular level and are likely to include improved exercise efficiency related to tighter coupling of muscular intracellular bioenergetics and mitochondrial function leading to improved mitochondrial efficiency, and/or improved muscle pH regulation and βm. Another novel mechanism may be related to changes in UCP3 content within the skeletal muscle to attenuate proton leakage across the mitochondrial membrane and improve efficiency of metabolic oxidation. Finally, with quite small performance benefits (1–2%) associated with LHTL it is possible that type II errors (false negatives) may be present in our search for mechanisms to explain an improvement in athletes' sea-level performance subsequent to altitude exposure. For instance, with a P value of 0.05 and power of 80%, a sample size of 64 athletes would be required for fully controlled study to detect a 1% improvement in performance if the within subject variation in performance is also 1% (http://sportsci.org/resource/stats/ssdetermine.html).

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