Physiological and Genomic Consequences of Intermittent Hypoxia
Invited Review: Adaptive responses of skeletal muscle to intermittent hypoxia: the known and the unknown

THOMAS L. CLANTON1 AND PAUL F. KLAWITTER 2
Departments of 1Internal Medicine (Pulmonary and Critical Care Division) and 2Emergency Medicine, Dorothy Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio 43210

Clanton, Thomas L., and Paul F. Klawitter. Invited Review: Adaptive responses of skeletal muscle to intermittent hypoxia: the known and the unknown. J Appl Physiol 90: 2476–2487, 2001.—Intermittent hypoxia (IH) describes conditions of repeated, transient reductions in O2 that may trigger unique adaptations. Rest periods during IH may avoid potentially detrimental effects of long-term O2 deprivation. For skeletal muscle, IH can occur in conditions of obstructive sleep apnea, transient altitude exposures (with or without exercise), intermittent claudication, cardiopulmonary resuscitation, neonatal blood flow obstruction, and diving responses of marine animals. Although it is likely that adaptations in these conditions vary, some patterns emerge. Low levels of hypoxia shift metabolic enzyme activity toward greater aerobic poise; extreme hypoxia shifts metabolism toward greater anaerobic potential. Some conditions of IH may also inhibit lactate release during exercise. Many related cellular phenomena could be involved in the response, including activation of specific O2 sensors, reactive oxygen and nitrogen species, preconditioning, hypoxia-induced transcription factors, regulation of ion channels, and influences of paracrine/hormonal stimuli. The net effect of a variety of adaptive programs to IH may be to preserve contractile function and cell integrity in hypoxia or anoxia, a response that does not always translate into improvements in exercise performance.

THE TERMS, INTERMITTENT HYPOXIA (IH), periodic hypoxia, and episodic hypoxia have been used to describe conditions of repeated, transient reductions in O2 that may play a role in triggering the responses to certain environmental or pathological conditions. When applied to the special case of tissues with a high metabolic rate relative to blood flow, such as cardiac or exercising skeletal muscle, intermittent tissue hypoxia can exist in the absence of reductions in arterial Po2 (PAO2). In such cases, capillary O2 supply fails to match metabolic demands, resulting in transient, localized hypoxia in the muscle cells or in the mitochondrial microenvironment.

We know very little about the level of cellular hypoxia in muscle required to trigger adaptive responses to IH. In fact, we know very little about IH. With a few exceptions (see, e.g., Refs. 12, 39, 83, 84), the actual stimulus within the muscle is rarely measured in vivo. Most studies have relied on descriptions of PAO2, inspired O2 fraction, or altitude as the stimulus modality. However, even tissue Po2 or myoglobin O2 saturation can only approximate the intracellular signal, particularly when hypoxia or ischemia are transient and coupled with varying contractile activity, where...
intermittent hypoxia (20). In contrast, dysoxia exists when the ability of COX, but metabolic adaptations to maintain ATP flux via aerobic metabolism are compromised function or even cell death. In contrast, when cells are exposed to an intermittent period of hypoxia (descending hyperbola), a sufficient hypoxic stimulus may initiate adaptive programs, but the time of exposure is insufficient to compromise function or induce cell death.

The hypoxic stimulus as viewed by the cell is complex. Two broad categories of cell hypoxia have been defined by Connett et al. (20): “adapted cell hypoxia” and “dysoxia” (Fig. 1). These definitions are adapted cell hypoxia (see text). Below this level, dysoxia results in eventual compromise in cell function and even cell death. Given sufficient time, dysoxia can lead to compromised function or even cell death in tissues of homeotherms, although certain heterotherms can survive dysoxia almost indefinitely (47). In trying to understand the adaptive responses to hypoxia, it is likely that different levels of PO2 are sensed by the cell. This may result in a graded recruitment of cell programs or simple enzyme responses that initiate unique strategies suited to optimizing ATP flux in specific O2 environments.

Why would the responses to IH differ from chronic hypoxia? In many cases, it is highly likely that the responses overlap, particularly when describing their effects on skeletal muscles. However, whereas long-term exposure to severe hypoxia can progress to cell injury and deterioration (14, 35), short-term exposures may be of sufficient amplitude to initiate adaptive responses while providing periods of recovery that avoid the injurious effects of O2 deprivation (Fig. 1). In addition, there may be unique physiological signals associated with transitions between hypoxia and reoxygenation, such as the formation of reactive oxygen species (ROS) (22, 95, 100, 102) or facilitation of sympathetic outflow (27, 92, 96) and other reflex pathways (67, 78), that can induce their own physiological responses.

There are a number of clinical or environmental conditions that are referred to as producing conditions of IH, including obstructive sleep apnea (OSA) (80), transient exposures to high altitude (with and without exercise) (34, 63, 110), intermittent claudication (12, 24, 53), exercise in diseases with compromised O2 delivery such as chronic lung disease or heart failure (33) events that obstruct placental blood flow (100), some unique conditions of myocardial infarction (32), and the diving responses of marine mammals (25). It is very unlikely that these diverse conditions result in identical cellular adaptations. In each, there are marked differences in the timing of hypoxic cycling, the length of exposure, and the degree of hypoxia with each cycle. Any of these variables could influence the pattern of response. There may also be important stimuli, such as PCO2, acidosis or alkalosis, and metabolite or nitric oxide (NO) accumulation, which vary depending on whether the hypoxia is induced by alterations in environmental PO2, apnea, or ischemia. However, all of these conditions are conceptually linked. Clearly, more specific definitions would be useful.

### MODELS OF IH

Table 1 summarizes the terms used in this review. The term IH will describe conditions of hypoxia in which PCO2 is allowed to remain at normal levels or to decrease with ventilatory responses to chemoreceptor stimulation. This condition is typical of brief exposures to altitude. Intermittent asphyxia (IA) will be used to describe conditions in which hypoxia is accompanied by hypercapnia, usually due to lack of effective ventilation, such as in OSA. Intermittent ischemia (IIsch) describes conditions in which lack of effective blood flow induces transient conditions of hypoxia and hyper-

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Table 1. Categories of intermittent hypoxia and direct applications

<table>
<thead>
<tr>
<th>Category</th>
<th>Arterial Po2</th>
<th>Arterial PCO2</th>
<th>pH</th>
<th>Metab</th>
<th>Short Cycle (&lt;5-min cycles)</th>
<th>Long Cycle (&gt;5-min cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent hypoxia (IH)</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>OSA</td>
<td>Intermittent altitude, intermittent exercise in altitude</td>
</tr>
<tr>
<td>Intermittent asphyxia (IA)</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>OSA, acute ventilatory failure</td>
<td>Diving mammals</td>
</tr>
<tr>
<td>Intermittent ischemia (IIsch)</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>Intermittent claudication, myocardial infarction, resuscitation, perinatal ischemic events</td>
<td>Severe peripheral vascular disease</td>
</tr>
</tbody>
</table>

OSA, obstructive sleep apnea; Metab, metabolites such as H+, CO2, nitric oxide, adenosine, and so forth. ↓, Decrease; ↑, increase.
capnia and retention of metabolites. These categories can be further refined with the descriptive terms short cycle and long cycle, which will refer to conditions of hypoxic cycling in periods of <5 min and >5 min, respectively.

Much of IH research regarding long-cycle IH has been driven by interest in understanding altitude physiology, particularly the potential for exercise at altitude to influence athletic performance (6, 57, 62, 63). The lines between IH and chronic hypoxia in these models are often obscured. For example, intermittent bouts of exercise at altitude, as might occur during mountain climbing, may represent a superimposed IH-like stimulus on top of a chronic hypoxia stimulus. Furthermore, some of the models of long-cycle IH provide a hypoxic stimulus for as long as 12 h/day, which may approach a chronic hypoxic environment (73–75). Still other models of continuous altitude acclimatization are performed at altitudes low enough that it is likely muscles are exposed to significant hypoxia only during exercise (57, 63).

In contrast, short-cycle oscillations in inspired \( \text{O}_2 \) are often used to model OSA (see, e.g., Refs. 17, 28, 92). This stimulus influences blood pressure (28), sympathetic control (92, 96), and ventilatory drive (53), responses mediated largely by oscillatory stimulation of the arterial chemoreceptors. There are differences, however, when considering such stimuli in muscle cells. Chemoreceptors are presumably capable of seeing the wave forms of \( \text{PaO}_2 \) with reasonable fidelity because of their high blood flow relative to metabolic rate. Such oscillations at muscle cells and muscle mitochondria are more likely damped by local metabolic autoregulation of blood vessels and capacitor-like behavior of \( \text{O}_2 \) storage systems (membranes and myoglobin). Therefore, in muscle cells, rapid oscillations in \( \text{PaO}_2 \) may be seen as much slower wave forms with lower amplitude.

ULTRASTRUCTURAL ADAPTATIONS

Although there is little conclusive evidence for ultrastructural and phenotypic changes in response to moderate IH, IIsch, and IA, there is some evidence in more severe conditions (18, 98). For example, no changes have been noted in intermittent exercise training at altitude (a model of IH) (62), in animal models of arterial insufficiency (41), in models of short-cycle IH (17), or in moderate peripheral occlusive disease in humans (98). However, as the severity of arterial occlusive disease worsens, the gastrocnemius muscle increases the percentage of type I myosin heavy chain (MHC) (98), characteristic of a shift to slow-oxidative fiber types. This is accompanied by decreases in type IIb MHC and eventual loss of type IIa MHC (98). Clyne et al. (18) reported somewhat contrasting results in this population using fiber typing. They observed a general loss of fiber cross-sectional area across the whole population of type I and II myocytes and attributed this to deconditioning. The changes in MHC seen in the gastrocnemius (98), however, are not particu-

larly consistent with deconditioning (77). They represent an interesting deviation from the response pattern seen in altitude acclimatization (35), disorders of gas exchange such as obstructive lung disease (60), or congestive heart failure (33, 106).

Chronic hypoxia induces quite specific ultrastructural modifications. There is a generalized muscle wasting and loss of cross-sectional area of all fiber types (14, 35). This is also reflected in a loss of muscle mass in the thigh (7) and no doubt contributes to a reduction in the maximum \( \text{O}_2 \) uptake measured in acclimatized subjects on return to sea level (15). It has been suggested that these responses are a form of muscle deterioration (14), but an argument can be made that the loss of mass is part of a programmed constellation of normal adaptive responses that minimize diffusion distances. For example, in some models of altitude acclimatization, capillary density appears to increase due to reductions in fiber cross-sectional area rather than angiogenesis (4, 63, 93). Mitochondrial volume density often decreases with altitude (45), but there are no apparent changes in the proportions of type I and II fiber types (35).

METABOLIC ADAPTATIONS

Metabolic responses to various forms of IH in intact mammals do not comprise a single pattern of adaptation. Furthermore, there are also no clear distinctions from chronic hypoxic stimuli. The combined complexity and universality of the response may reflect the evolutionary importance of hypoxic adaptation, as primitive organisms developed responses comprising a variety of long- and short-term strategies to sustain energy flux in widely varying \( \text{Po}_2 \). Unfortunately, the data we have to work with in understanding metabolic adaptations of intact, higher organisms are restricted to single, pooled samples of in vitro enzyme activities, high-energy phosphates, and substrate/metabolite concentrations from heterogeneous muscles. Clearly, a great deal of noise is inherent in these measurements, and there is much disagreement in the literature. However, some interesting patterns of response emerge.

Oxidative metabolism. In models of both chronic hypoxia and IH, there appear to be biphasic responses of metabolic enzyme activities involved in the Krebs cycle and possibly electron transport. Figure 2A summarizes data from a large number of studies (34–36, 44, 45, 62, 73–75, 86, 110) on animals and humans subjected to both intermittent and chronic hypoxia. Changes in oxidative enzyme activity are expressed as percent changes in Krebs cycle enzymes [usually citrate synthetase (CS)] from controls. We have combined both IH and chronic hypoxia in this analysis for purposes of discussion. As shown, whether the hypoxic stimulus is intermittent or chronic, very low levels of inspired \( \text{O}_2 \) exposure result in a general loss of Krebs cycle enzyme activity. In contrast, at more modest levels of hypoxia, somewhere above an atmospheric \( \text{Po}_2 \) of 80–100 Torr, stimulations of aerobic enzyme activities are evident (34, 35, 62), particularly when coupled with exercise.
Please note that considerable liberties were taken in generating the relationship in Fig. 2. Not only are there widely varying intermittent and chronic hypoxia paradigms and species but variables that have been demonstrated to influence enzyme activity were not factored in. These include days of exposure to either IH (44, 74, 75) or chronic hypoxia (79), the level of exercise conditioning during exposure (34, 62, 63), and age (73). Nevertheless, the relationship seems to suggest a biphasic pattern of response in which strategies promoting stimulation of oxidative capacity are reversed at a critical PO2.

In contrast, Fig. 2, B and C, was obtained from a study of patients with intermittent peripheral arterial insufficiency, a clinical condition of IIsch during exercise. In this condition, metabolic adaptations are less clear but also seem to follow a pattern of predominant facilitation of oxidative enzyme activity in moderate disease, which then declines in more severe disease. Patients shown in Fig. 2, B and C, had conditions ranging from modest intermittent claudication (stage I) to severe resting ischemia (stage III) (11). Note that, in general, both CS and COX activities are increased. This observation is consistent with some other patient literature (59) but not all (18). For example, Clyne et al. (18) reported reductions in oxidative enzyme activity that worsened with disease. Differences in results between studies may reflect levels of disease or the amount of exercise undergone at a given level of disease. For example, increases in CS activity have been seen in models of hindlimb arterial ligation in rats when combined with exercise (24) but not in similar studies in which the hindlimbs were not exercised (41).

In Fig. 2C, note that, after a rise in COX at low to moderate disease, there is a trend toward reduction of activity in severe disease, reminiscent of the biphasic response seen for Krebs cycle enzymes in Fig. 2A. This brings up several points. First, oxidative enzyme activities do not always change in parallel. Although mitochondrial enzymes involved in beta oxidation of fatty acids usually increase (11, 36, 59, 86) or decrease (45) in parallel with CS, it has been recently shown that, in some cases, CS and COX activities may change in opposing directions (89). This suggests an uncoupling in the regulation of Krebs cycle and oxidative phosphorylation under some conditions (88). It is suggested that this may be an influence of aging because young rats exposed to severe IH show decreases in CS activity, whereas COX activity stays the same or increases. In contrast, old rats exhibit parallel decreases in CS and COX (73), as if the genes regulating compensatory responses of COX could not be upregulated in the aged animal. Regardless, the enzymatic and genetic responses of mitochondrial enzymes are probably more complex than was previously realized (88).

We cannot assume that responses to IIsch are the same as those to IH. The nature of the stimulus is different. For example, in moderate peripheral insufficiency, hypoxia occurs only when coupled with exercise, because the limb tissue PO2 is normal (12, 58). In addition, other factors such as the buildup of metabolites in ischemia can have independent influences on metabolic pathways and intracellular O2 sensors, as will be discussed later. The old hypothesis that tissue hypoxia is a “common trigger” for metabolic adapta-
tions in physical training, ischemia, and hypoxia (44) is likely an oversimplification of a complex network of responses.

**Glycolytic enzymes.** Characteristic changes in lactate dehydrogenase (LDH) activity using a variety of models of chronic hypoxia and IH exposure (35, 45, 73, 74, 75, 110) are shown in Fig. 3A. LDH is discussed here because it is one of the more frequently measured enzyme activities related to glycolysis, and data for other enzymes such as phosphofructokinase (PFK) are less abundant in the literature. In addition, the ratio of LDH to CS has been used as a general indicator of adjustments to relative anaerobic/aerobic potential (43). The same caveats apply for this summary of data as in Fig. 2. However, generally, in most studies of humans exposed to chronic hypoxia, there is little or no appreciable shift in LDH activity (35, 45, 86). Other enzyme activities in the glycolytic pathway such as PFK may actually drop (36) or increase (45), depending on the experiment. In contrast, in long-cycle IH models in rats, large increases of LDH are apparent, which are exactly out of phase with the decreases seen in aerobic enzyme activities shown in Fig. 2A. Resultant changes in LDH/CS suggest that, in severe IH, there is a shift to greater anaerobic/aerobic potential.

The adaptations of glycolytic enzymes in arterial insufficiency are less clear and remain controversial. Changes in LDH and PFK, shown in Fig. 3, B and C, are from the same study shown in Fig. 2, B and C. Progressive reductions in LDH are seen, with biphasic-like responses of PFK very similar to those of CS (Fig. 2A). However, later studies from the same authors in patients with moderate intermittent claudication (approximately equivalent to stage I) have shown no such changes in these enzymes (59). Furthermore, Clyne et al. (18) observed increases in anaerobic enzyme activities. In rat models of femoral artery ligation, PFK activity is upregulated in the first 7 days but returns to normal after 8–10 wk and LDH is essentially unchanged throughout (41). In a rat model in which femoral ligation is coupled with electrically stimulated exercise (simulating intermittent claudication), the levels of PFK were essentially unchanged over 4–6 days (24). It is interesting to speculate that the differences in responses between studies or between patient groups again reflects the combined stimulus of exercise and local hypoxia, which would be required to induce a substantial IH stimulus. It should be noted that these alterations (or lack thereof) in glycolytic and oxidative enzyme activities may be accompanied by simultaneous changes in fiber distribution in these samples (18, 98). Pooled samples from muscles with type II fiber atrophy may hide relative increases in glycolytic capacity of the remaining type I phenotypes. This underscores the difficulty in interpreting global muscle responses from pooled samples when we have little information of what is happening in individual cells.

**Glucose and glycogen.** The transport of glucose, its phosphorylation, and the ability to store and utilize glycogen are other adaptations of known importance to the cell’s response to acute hypoxia. In eleven of the samples of IH and continuous hypoxia illustrated in Figs. 1 and 2, nine showed increases or trends suggesting elevations in hexokinase (HK) activity (see text for references). The solid line is the linear regression of all points; the dashed line is the hypothetical intersection with normoxia at sea level. B: lactate dehydrogenase (LDH) activity in biopsies from patients with peripheral arterial insufficiency. [Redrawn from Ref. 11.] C: phosphofructokinase (PFK) activity in progressive states of peripheral arterial insufficiency. [Redrawn from Ref. 11.]

**Fig. 3.** A: summarized data from a sample of studies on glycolytic enzymes in rats and humans during intermittent or chronic hypoxia (see text for references). The solid line is the linear regression of all points; the dashed line is the hypothetical intersection with normoxia at sea level. B: lactate dehydrogenase (LDH) activity in biopsies from patients with peripheral arterial insufficiency. [Redrawn from Ref. 11.] C: phosphofructokinase (PFK) activity in progressive states of peripheral arterial insufficiency. [Redrawn from Ref. 11.]

Glucose and glycogen. The transport of glucose, its phosphorylation, and the ability to store and utilize glycogen are other adaptations of known importance to the cell’s response to acute hypoxia. In eleven of the
least one model of arterial insufficiency in the rat, there are marked increases in HK activity in mixed, slow-twitch, and fast-twitch muscles of the hindlimb after 7 days of femoral ligation (41). However, in human arterial insufficiency, no remarkable changes in HK have been observed (11). It has been suggested that the increase in HK activity is related to greater dependence on glucose with adaptation to hypoxia (9).

We simply do not have enough information about glucose transport in models of IH to draw significant conclusions. It may be an interesting area of research because glucose transport in hypoxia involves pathways that are activated somewhat differently from insulin or contractile-dependent changes in uptake (107, 109). Furthermore, hypoxia-induced transcription factors (HIF-1α) upregulate glucose transport proteins (Glut1 and Glut3) (10). It is worth mentioning that hypoxia-induced glucose uptake during contractions is, in part, adenosine dependent, which means that important regulating signals might be different from those in IH, in which washout of adenosine would be high (52), vs. those in IIsch or arterial insufficiency, in which adenosine would accumulate in the tissue (21).

Although there has been no study to our knowledge of the influence of IH on glycogen utilization and storage, there have been recent studies in hearts exposed to brief periods of IA (61). Withdrawal of mechanical ventilation and clamping of the airway for 90-s periods, separated by 5-min intervals of ventilation, caused activation of glycogen synthase and rapid accumulation of glycogen immediately after hypoxia (61). The signal for this alteration appears to be directly related to glycogen breakdown during hypoxia (61). It is unknown whether more long-term adaptations occur to glycogen synthesis in response to IH.

The "lactate paradox." Exercise during acute hypoxia results in stimulation of lactic acid production compared with the same exercise performed during normoxia. However, after acclimatization to altitude, the lactic acid production is markedly attenuated, in both muscle and blood compared with acute exposure (5, 36, 37, 62). This phenomenon has been called the lactate paradox (42), but it may not be unique to chronic hypoxia exposure or altitude acclimatization. Similar responses are seen in conditions of IA (105). For example, in patients with OSA, maximal lactate concentrations in exercise are ~60% of those seen in control subjects (105) in a condition in which elevated diastolic pressures, normal maximum heart rates, and a propensity toward deconditioning are prevalent. In conditions of IIsch, such as exercise with arterial insufficiency, lactate measurements in muscle achieve high levels during exercise (13). This may reflect metabolite accumulation because, when measurements of rates of release are calculated, muscles undergoing combined stimulation and ligation show decreased lactate release compared with stimulation in the unligated, contralateral leg (23). It seems reasonable to speculate that lactate paradox may be a generalized phenomenon, common to many forms of adaptation to hypoxia.

A number of mechanisms have been suggested to account for lactate paradox. These include 1) decreased glycolytic flux (8, 2) decreased glycogen storage (105), 3) changes in buffering capacity (14, 63), 4) changes in efficiency of muscle contraction (105), and 5) improved coupling between oxidative phosphorylation and glycolytic flux (36). The mechanism in OSA or arterial insufficiency has not been evaluated, but it is well known that in patients with OSA and in models of IH there is an overall stimulation of the sympathetic outflow, resulting in systemic hypertension (27, 66). The increased circulating epinephrine could potentially inhibit glycogen synthetase (29). Because it has been estimated that 99% of the glucose that is made available for glycolysis during exercise comes from glycogen stores (54), reduction of glycogen stores could depress glycolytic flux during exercise. Exercise responses in subjects acclimatized to altitude show a glycogen sparing effect that would also be consistent with this mechanism (36). The role of buffering capacity on glycolytic control is related to the fact that one of the primary rate-limiting enzymes in glycolysis, PFK, is inhibited by acidosis (97). In altitude acclimatization, there is a loss of buffering capacity of the blood due to HCO₃⁻ excretion as the kidneys compensate for respiratory alkalosis. This may be compensated somewhat by increases in muscle tissue buffering capacity (63). In acclimatized individuals, as lactic acid is formed during exercise, there is a greater relative drop in blood pH for a given amount of lactic acid produced. This, in turn, could conceivably result in decreased cell pH, thus self-limiting glycolysis by inhibition of PFK (97). It is unlikely that this mechanism is predominant in IH or OSA because long-term changes in HCO₃⁻ are not apparent (105); however, cell buffering capacity was not measured.

OTHER ADAPTIVE RESPONSES

Ion transport. Recently, Green et al. (34) have shown that, when intermittent exercise training is conducted in hypoxia, there is a downregulation of Na⁺/K⁺-ATPase pump expression in limb muscle, which is in contrast to exercise training alone (34). The significance of this finding is not known, but the important role of ion gradients in sustaining membrane potential and the possible influence of extracellular K⁺ on contractile function during high-frequency fatigue have been emphasized (70). In heart muscle, enzyme studies on membrane-bound Mg²⁺, Ca²⁺, and Na⁺/K⁺-ATPases showed no change in maximum activity but a lowering of the Kₘ, signifying an increased enzyme affinity for the substrate (112). This effect could slow the rate of decline of enzyme activity in severe hypoxic exposures.

In a recent study of short-cycle IH in the rat (17), marked changes in twitch contraction characteristics were observed in isolated diaphragm with no changes in maximum force generation. Increases in passive tension (contracture), normally observed during exposure to anoxia, were completely inhibited in the IH-
treated rats. Although there are many potential mechanisms for these findings, one intriguing possibility is that membrane ion channel and pump functions were altered by IH, affecting ion movements during normoxic conditions and preserving membrane potential and preventing Ca\(^{2+}\) leakage during hypoxia.

**O\(_2\) transport.** There is very little information regarding changes in myoglobin content in models of IH or adaptive changes in the capacity to deliver O\(_2\). Humans native to high-altitude environments show myoglobin contents in the sartorius \(\sim\)16% above those of natives of similar ethnicity living at sea level (82). How rapidly these changes occur or whether they occur in IH are not known. At 5,100 m, it requires 14 wk for myoglobin to be significantly elevated in guinea pig soleus muscle (93).

Recent studies have also hypothesized that O\(_2\) distribution in highly metabolic tissue may be affected by NO generated in the capillary endothelium (101) or possibly from the myocytes themselves. Gradients of NO, radiating from the vessel, are believed to inhibit mitochondrial respiration in locations of high PO\(_2\), close to the vessel, thus preserving O\(_2\) gradients for mitochondria at greater distances (101). This effect could be amplified in acute ischemia because of NO accumulation. Furthermore, it could also be amplified in chronic hypoxic exposures, as it has been shown that one of the adaptive responses to chronic hypoxia in muscle is upregulation of endothelial and neuronal isoforms of nitric oxide synthase (48). However, this concept has not been tested in IH and cannot explain improved function in anoxia (17).

**Autocrine/paracrine influences.** Global IH has a number of influences on circulating hormones and local metabolites that could play important roles in adaptive responses of muscle to IH. Little is known about the significance of these signals, but the issue is raised here to illustrate the complexity of the stimulus and to inspire future work. The possibility that IH-induced elevations in sympathetic tone, specifically epinephrine release, can affect glycogen storage has been mentioned. However, epinephrine can also stimulate cAMP in muscles, which could have secondary effects on multiple metabolic and cell signaling pathways (see, e.g., Refs. 10, 16, 30, 69, 79).

Sleep apnea has also been found to induce the release of endogenous digitalis-like factors that directly inhibit Na\(^+\)-K\(^+\)-ATPase (71). Leptin levels are increased in patients with OSA, beyond the levels measured in similarly obese individuals (78). Leptin is produced by adipose tissue and is involved in a number of physiological feedback loops, including inhibition of appetite (90) and stimulation of metabolism (46). Atrial natriuretic peptide (ANP) release is increased in patients with OSA (56), and, in animal models of short-cycle IH, mRNA for both A and B type ANP is increased in right and left ventricles (55). Other interesting paracrine effects may arise from endothelial cells. Hypoxic endothelial cells release low-molecular-weight peptides in response to hypoxia that directly inhibit the unloaded shortening velocity of skinned soleus muscles and depress in vitro cardiac myosin ATPase activity (91). It is interesting to note that cardiac myofibril ATPase activity is reduced in animals exposed to intermittent altitude (76). Furthermore, adenosine, released during hypoxia and ischemia, can have many local paracrine and autocrine effects by stimulation of adenosine receptors on the cell membrane. Of particular importance may be its role as a “preconditioning” stimulus (19), as discussed below.

**Overall effects of adaptation.** The symphony of structural and enzymatic changes that occur in skeletal muscle can act as a reflection of hypoxia-induced disorders of metabolic regulation or could represent specific strategies designed to preserve contractile function and cell viability. Interestingly, this question has rarely been addressed in skeletal muscle. Recent results in isolated diaphragm favor the latter theory (17). After a 10-day exposure to short-cycle IH, contractile function of the diaphragm was greatly improved during anoxia and on reoxygenation compared with control animals (17). Resistance to hypoxia as an outcome variable could be differentiated from adaptive responses to exercise training, since no effects on strength (maximum force) or endurance of the diaphragm were seen (17).

Similar observations have been made from experiments in cardiac muscle in which intermittent exposure to hypoxia may reduce the risk of coronary heart disease and prevent the onset of arrhythmias (3, 81, 113). Cardiac myocytes exposed to 1% O\(_2\) for 48 h (which is described as providing full oxygenation) develop remarkable tolerance to subsequent hypoxias, showing delays in ATP depletion rigor and improved morphological recovery after reoxygenation (94).

### CELL SIGNALING MECHANISMS OF ADAPTATION IN IH: FUTURE DIRECTIONS OF RESEARCH

**O\(_2\) sensors.** Perhaps of all tissues in the body, skeletal muscles see the widest fluctuations in O\(_2\) demand and O\(_2\) delivery. Therefore, it would seem likely that they have developed sophisticated mechanisms for sensing changes in local O\(_2\) to which they could respond by regulating O\(_2\) demand and uptake, adjusting metabolic pathways and possibly initiating cell remodeling. Although the biology and biophysics of O\(_2\) sensors has been a proliferative area of research, it has not been studied in detail in skeletal muscle. A comprehensive review of O\(_2\) sensor biology exceeds the scope of this review, but molecules such as membrane NADPH oxidases, mitochondrial COX, a variety of K\(^+\) channels, metal-containing heme groups, or iron-sulfur clusters have all been implicated as molecular O\(_2\) sensors in various tissues and organisms (see, e.g., Refs. 10, 16, 30, 69, 79).

One apparent O\(_2\) sensor, which was recently described in skeletal muscle and which may have particular relevance to models of IH, is the Ca\(^{2+}\) release channel or ryanodine receptor. Eu et al. (26) have demonstrated that the ryanodine receptor is dramatically stimulated by dropping intracellular PO\(_2\) from ambient pressures to \(\sim\)10 Torr. Although the mecha-
nism is not completely worked out, it appears as though a specific thiol is nitrosylated in low O2. The nitrosylation occurs by reactions of available thiols with NO, but the local availability of NO and the responsiveness of the channel to nitrosylation are set by the Po2, i.e., the “responsiveness of the channel is thus tuned by the Po2” (26). This kind of a sensor could be activated by brief exposures to hypoxia and might be activated differently in conditions of ischemia (where NO dwell times are extended) or hypoxia-reoxygenation, which would favor an oxidizing environment. Such a mechanism could compensate for loss of contractile function due to metabolite feedback or low phosphorylation potential.

Reactive oxygen as a signaling molecule in hypoxia. In recent years, a greater appreciation has emerged regarding the role of ROS and free radicals as signaling molecules in gene regulation (1). In sufficient concentration, they can, of course, cause a variety of degrees of oxidative damage. Although not generally appreciated, cell hypoxia, or ischemia alone, can result in the formation of low levels of ROS when the NADH/NAD+ reduction state of the cell is high and sufficient O2 is still available for single electron transfer. This phenomenon has been demonstrated most convincingly in isolated cardiac myocytes (22), but other studies in isolated hearts have drawn similar conclusions (72). Our laboratory observed such responses in isolated rat and mouse diaphragm as well (unpublished observations). The exact mechanism by which hypoxia induces ROS formation has not yet been identified in muscle. During reoxygenation, there is a secondary increase in ROS formation that is typical of hypoxia-reoxygenation and ischemia-reperfusion in a variety of organs (see, e.g., Refs 31, 100, 104). That ROS have functional effects during hypoxia and reoxygenation in skeletal muscle is illustrated by the fact that antioxidant administration (particularly superoxide scavengers) results in marked improvements in contractile function during and after hypoxia (64). ROS formation is likely amplified by conditions of repeated hypoxia-reoxygenation exposure, at least in some tissues (see, e.g., Refs. 25, 31, 95, 100). However, in heart muscle, repeated exposures result in an attenuation of ROS formation (104). One striking example of the detrimental effects of repeated hypoxia-reoxygenation is revealed in the effects on neonatal brain cell viability after intermittent uterine ischemia-reperfusion, a model of eclampsia, or episodic contractions of labor (100). Repeated exposures cause marked oxidant injury and cell death in brain neurons, which is attenuated by antioxidant treatments. Indirect evidence in intact skeletal muscle that IlSch or IH produces ROS also comes from experiments using microdialysis of the redox state of glutathione (95). IlSch reduces the intracellular glutathione stores in muscle and results in active extrusion of oxidized glutathione from the tissue, thus reducing antioxidant defenses (95).

Is IH a sustained preconditioning stimulus? An attractive hypothesis regarding a potential pathway of adaptation is that IH stimuli induce a continuous state of preconditioning, protecting the muscle from subsequent exposures to ischemia. Preconditioning is a concept that arose in the cardiac literature (65) and has become a very large topic in the cardiobiology literature, well beyond the scope of this review (see reviews, Refs. 19, 108). However, several of the mediators discussed previously are known to play important roles in preconditioning. For example, ROS have been implicated by the fact that antioxidant treatments administered during ischemic preconditioning abolish the protective effects of preconditioning in heart muscle (103). Furthermore, preconditioning inhibits ROS formation in subsequent periods of ischemia-reperfusion (104). Likewise, treatment with adenosine induces an equally strong preconditioning-like influence in heart muscle that can be blocked by adenosine antagonists (87). These mediators may work in synchrony by activating protein kinase C pathways (19), upregulating antioxidant defenses (111), upregulating stress proteins (51), and the activating mitochondrial K+-ATP channels (87). Interestingly, the protective influence of chronic altitude exposure or chronic hypoxia appears to work by mechanisms that are additive to those induced by brief periods of ischemic preconditioning, suggesting that preconditioning and long-term hypoxia operate through parallel pathways (2, 99).

Hypoxia-induced gene expression in muscle. Very little is known about how IH influences gene expression in muscle. Recent studies of responses to acute hypoxia and exercise, however, shed some light on possible pathways of adaptation (38, 84, 85). Gustafsson et al. (38) found that, in response to single exercise sessions with various levels of restricted blood flow (IlSch), muscle hypoxia-inducible factors (HIF-1α, HIF-1β) and vascular endothelial growth factor (VEGF) are dramatically increased. The HIF-1α and -β transcription factors are necessary for VEGF expression and are activated within a physiological Po2 range consistent with exercising muscle (49). Interestingly, the level of upregulation was correlated to the femoral venous lactate concentration, suggesting a relationship between tissue O2 uptake/delivery and gene expression. Richardson et al. (84) demonstrated similar findings in a model of hypoxia with exercise. However, in this study, no significant amplification of VEGF mRNA was seen when hypoxia was coupled with exercise compared with exercise in room air. Furthermore, no relationship was observed with the level of muscle oxygenation (84). Interestingly, after a period of exercise conditioning, the increase in VEGF in response to exercise was greatly diminished (85). This was used to support the idea that the VEGF response system is tightly regulated. In the trained state, when presumably capillarization and oxygenation are optimized, the response appears to be attenuated by negative feedback mechanisms. Would similar adaptive responses occur with long-term exposure to IH?
SUMMARY AND CONCLUSIONS

The primary distinguishing feature of IH, as it applies to skeletal muscle, is the presence of periods of recovery, providing windows of time for anabolic responses while avoiding the detrimental effects of long-term O₂ deprivation (Fig. 1). At this time, there are no clear qualitative distinctions between the metabolic adaptations that occur between IH and chronic hypoxia in muscle. So far, experiments are consistent with the hypothesis that differences in response patterns between varying experimental paradigms predominately reflect the magnitude and perhaps the duration of the hypoxic stimulus. Different levels of hypoxia may initiate different adaptive programs, possibly from stimulation of different molecular sensors with varying O₂ affinities. Responses may be further influenced by exercise, either by direct effects on tissue oxygenation or intersection with other cell signaling networks. The predominant adaptive strategies at a given hypoxic exposure appear to be functionally related to the cell's capacity to extract available O₂. For example, low levels of hypoxia or IIsch (particularly when coupled with exercise) facilitate aerobic metabolism, whereas more severe hypoxia (perhaps dyoxia) may favor upregulation of glycolysis. Very extreme, prolonged hypoxia can initiate catabolic processes that may purposefully be designed to improve O₂ diffusion distance. The inhibition of lactic acid production appears to be a common feature of most forms of hypoxic adaptation, including IH, but it is possible that in different models, the strategies used to accomplish this are different. Although the inhibition of lactate production has sometimes been referred to as an “impairment to metabolism” (105), it is possible that it represents a mechanism by which the contractile and glycolytic inhibition associated with acidosis is prevented. It may also function to preserve the predominant flow of energy substrates to oxidative phosphorylation by improved coupling with glycolysis. Other pathways of adaptation, such as changes in the activities of membrane pumps or channels, changes in mechanisms of O₂ storage or distribution, or influences of autocrine or paracrine signals, are not well defined.

Clearly, our understanding of IH or even chronic hypoxia in skeletal muscles is presently sketchy. It is our view that studying models of IH may reveal new information about hypoxic adaptation that would be distorted in studies of chronic exposure. In addition, studies of IH may make it possible to distinguish components of exercise adaptation that might be attributed to hypoxic signaling vs. pathways stimulated by contraction activity. Although considerable speculations regarding possible involvement of ROS, preconditioning stimuli, O₂ sensors, hormonal/paracrine signaling, channel function, and growth factor gene expression were included in the review, it is our hope that this will stimulate interest and discussion in this area of research.

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