Chapter 18

THE ROLE OF HIF-1 IN HYPOXIC RESPONSE IN THE SKELETAL MUSCLE

Steven Mason and Randall S. Johnson
Molecular Biology Section, Division of Biological Sciences, UC San Diego, San Diego, California, USA.

Abstract: During endurance training, exercising skeletal muscle experiences severe and repetitive oxygen stress, and the muscle's ability to cope with and improve its function through that stress is central to its role in the body. The primary transcriptional response factor for hypoxic adaptation is hypoxia inducible factor-1α (HIF-1α), which upregulates glycolysis and angiogenesis in response to low levels of tissue oxygenation. To examine the role of HIF-1α in endurance training, we have created mice specifically lacking skeletal muscle HIF-1α and subjected them to an endurance training protocol. We found that only wild type mice improve their oxidative capacity, as measured by the respiratory exchange ratio; surprisingly, we found that HIF-1α null mice have already upregulated this parameter without training. Furthermore, untrained HIF-1α null mice have an increased capillary to fiber ratio, and elevated oxidative enzyme activities. These changes correlate with constitutively activated AMP-activated protein kinase in the HIF-1α null muscles. Additionally, HIF-1α null muscles have decreased expression of pyruvate dehydrogenase kinase I, a HIF-1α target that inhibits oxidative metabolism. This data demonstrates that removal of HIF-1α causes an adaptive response in skeletal muscle akin to endurance training, and provides evidence for the suppression of mitochondrial biogenesis by HIF-1α in normal tissue.

Key Words: skeletal muscle, endurance exercise, oxidative capacity, HIF

INTRODUCTION

The greatest challenge facing skeletal muscle is the need to match ATP production with energy demand during exercise. As exercise intensity rises, the demand for ATP increases, and more rapid and efficient ways of producing ATP are required. The pathways leading to ATP production during exercise can be divided into two major categories: aerobic (oxygen requiring) and anaerobic (oxygen independent). During exercise, a muscle must balance the input of both aerobic and anaerobic metabolism to meet energy demands, and the balance between the two is determined by the type, intensity, and duration of exercise (5). Endurance exercise relies primarily on aerobic metabolism for ATP generation, meaning the muscle must the available oxygen to produce much-needed ATP. The difficulty of this task is compounded by the availability of

Hypoxia and the Circulation, edited by R.C. Roach *et al.*
Springer, New York, 2007.

oxygen to the muscle, which can change greatly from rest to exercise. During exercise in normoxia, the partial pressure of oxygen in the muscle has been measured at 3.1 mm Hg, even though oxygen in the inspired air has a partial pressure of 160 mm Hg, and oxygen in the capillaries in the muscle has a partial pressure of 38 mm Hg (55). This low level of oxygen during exercise necessitates a mechanism to enable the muscle to maintain optimum performance.

THE CELLULAR HYPOXIC RESPONSE AND HIF-1α

The primary oxygen response factors within a cell are the transcription factors of the Hypoxia Inducible Factor (HIF) family, HIF-1, HIF-2 and HIF-3. Only two of these members, HIF-1 and HIF-2, have been characterized appreciably. Of those two, HIF-1 is the more ubiquitous member (67), as the induction of HIF-2 protein under hypoxia is limited to certain cell types within tissues (79).

First purified and sequenced in 1995, HIF-1 is a heterodimeric protein composed of two basic helix-loop-helix-PAS transcription factors: the aryl hydrocarbon nuclear receptor (ARNT, also referred to as Hypoxia Inducible Factor-1β), and HIF-1α (75, 77). While HIF-1α and ARNT are each constitutively expressed and translated, ARNT protein levels are relatively stable but HIF-1α protein levels are regulated primarily by the availability of oxygen to the cell. Under normoxic conditions, HIF-1α protein is hydroxylated by members of a family of prolyl hydroxylases on two conserved proline residues in its oxygen-dependent degradation domain (ODD) (6, 14). This hydroxylation enables recognition of HIF-1α by an E3 ubiquitin ligase complex, of which the von Hippel Lindau (VHL) protein is the primary factor responsible for recognizing and binding to hydroxylated HIF-1α (29, 30). The hydroxylation of HIF-1α at its proline residues is essential for this interaction as their mutation results in less binding of VHL with HIF-1α (14). Further verification of the importance of the proline residues comes from other studies looking at manipulation of the ODD. Wholesale deletion of the ODD results in a stable HIF-1α protein and HIF-1 target gene activation, and fusion of the ODD to a normally oxygen-insensitive protein makes that protein oxygen sensitive (28). The interaction of HIF-1α with VHL results in ubiquitylation of HIF-1α, and targeting of HIF-1α to the 26S proteasome for degradation (9). This regulation of HIF-1α protein through hydroxylation is quite strict; the half-life of new HIF-1α protein under normoxia has been demonstrated to be as short as five minutes (28).

When oxygen concentration drops, and cells and tissues become hypoxic, the hydroxylation of HIF-1α is blocked, resulting in decreased interaction between HIF-1α and VHL (30). As a result, HIF-1α protein is stabilized, allowing it to dimerize with ARNT and turn on transcription of target genes. The oxygen sensing machinery that so tightly regulates HIF-1α under normoxia is quite sensitive to inhibition by hypoxia; hypoxic cells begin accumulating HIF-1α protein within 2 minutes of hypoxic exposure (31). In vivo, the sensitivity of cells to hypoxia is tissue-specific. In work with mice exposed to normobaric hypoxia, Stroka et al. (67) saw that brain tissue begins accumulating HIF-1α protein when inspired oxygen is dropped to 18%, while kidney and liver

only respond to more severe hypoxia. Additionally, the authors found stable HIF-1α protein under normoxia in skeletal muscle, showing that some tissues have the ability, and need, to accumulate HIF-1α protein independently of hypoxia. This finding was recently repeated by Pisani and Dechesne (54), who additionally showed that normoxic HIF-1α stability in the muscle is dependent on fiber type. Muscles that are composed primarily of type II fast twitch fibers have a higher level of HIF-1α protein at rest in normoxia than muscles with a higher proportion of type I fibers.

Once HIF-1α is stabilized, it interacts with ARNT, forming the HIF-1 complex. This enables HIF-1 to recognize hypoxia responsive elements (HRE) in the promoters and/or enhancers of genes in the nucleus. The HRE is a short consensus sequence that HIF-1 binds to in order to upregulate transcription of target genes (44, 76). Once activated, the transcriptional response of HIF-1α to hypoxia enables cells to cope with oxygen stress while working to increase oxygen delivery (65). To help cells and tissues survive oxygen stress, HIF-1α upregulates transcription of genes that amplify glycolysis and glucose transport into the cell. Genes in this category include glucose transporters 1 and 3 (GLUT1, GLUT3), as well as the glycolytic genes hexokinase I and II (HKI, HKII), phosphoglycerate kinase 1 (PGK1), and lactate dehydrogenase A (LDHA), among others (64). In order to increase oxygen availability, HIF-1α coordinates a response that increases oxygen delivery to the hypoxic region. Two key transcriptional targets for this function are vascular endothelial growth factor (VEGF), and erythropoietin (EPO) (64). Other HIF-1α target genes include genes involved in cell cycle and apoptosis signaling, however, the role of HIF-1α in the cellular proliferation/survival response is not completely understood. In addition to the multitude of genes identified as having HREs in their promoters (meaning they can be directly regulated by HIF-1α), many more genes have been shown to have expression patterns correlating with HIF-1α activity, indicating that they are also directly or indirectly regulated by HIF-1α. GLUT4, the primary muscle glucose transporter, falls into this category (66). New HIF-1α targets are continually being discovered as the understanding of how HIF-1α helps cells and tissue respond to hypoxia grows.

Our research has shown that loss of HIF-1α can have profound effects on cells and tissues. The primary result of the loss of HIF-1α is that cells are unable to upregulate HIF target genes in response to hypoxia. This leads to a failure to upregulate glucose transport and glycolysis, resulting in decreased ATP levels during hypoxia (63). Surprisingly, this failure extends to normoxia as well for some cell types, as macrophages lacking HIF-1α have as little as 15-20% of the ATP content under normoxic conditions as control macrophages (10). HIF-1α is also essential for development, where local hypoxia results from the lack of an established vascular system. In evidence of this, mice lacking HIF-1α in their germ line die *in utero* due to defects in cephalic vascular formation and defective neural fold formation (58).

Another important role for HIF-1α has been found in tumor growth and development. Solid tumors become hypoxic as they grow larger, and tumors forming following inactivation of the VHL tumor suppressor protein are aggressive and well vascularized (38), leading to the hypothesis that HIF-1α is a positive factor in tumor development. To that end, we have shown that solid tumors lacking HIF-1α do not grow as rapidly as normal tumors, indicating that this is indeed the case (59). Furthermore, we recently

found that deletion of HIF-1α in mammary epithelial tissue results in delayed tumor onset, retarded tumor growth, and reduced pulmonary metastasis in a breast cancer model system (39).

Tissue and cell type-specific deletion of HIF-1α has shown HIF-1 signaling to be integral in many different places in the body. Deletion of HIF-1α in chondrocytes results in bone deformities and abnormalities in the trachea due to increased chondrocyte growth (62), while in myeloid cells, loss of HIF-1α reduces their mobility and invasiveness, and their ability to kill bacteria (10). Combining the results of these studies shows that the hypoxic response through HIF-1α plays an important role in development, disease, and homeostasis.

MUSCULAR RESPONSE TO ACUTE ENDURANCE EXERCISE

As referenced above, skeletal muscle experiences a drop in intramuscular oxygen during exercise, leading to a hypothesis for a possible role for HIF-1α in the muscle during and following exercise. Surprisingly, however, little research has been done looking directly at HIF-1α function in the muscle prior to our studies.

In the muscular response to exercise, several changes occur that are likely mediated by HIF-1α. Due to the increased demand for oxygen in the muscle, both the body and the skeletal muscle undergo several acute performance-oriented changes. These changes have the goal of increasing oxygen delivery to the muscle and improving its metabolic capabilities. Since an acute exercise bout is too short of a time period to allow for vascular remodeling or a significant increase in red blood cell content, one of the primary ways exercising skeletal muscle receives greater oxygen delivery during exercise is through increased blood flow to the skeletal muscle. This is accomplished through two main pathways: a decrease in blood flow to non-exercising tissues (i.e., the kidney and spleen) and increased blood flow to the skeletal muscle itself (56). In addition to increased oxygen delivery, the greater blood flow also allows for increased metabolite delivery to and waste clearance from the exercising muscle.

The metabolic changes in exercising muscle serve to increase ATP production while minimizing the impact of non-essential ATP consuming pathways. A key protein that helps the muscle accomplish this is the AMP-activated protein kinase (AMPK). Exercise, and the resulting increase in ATP consumption, causes an increase in the AMP to ATP ratio. AMP then binds with AMPK, making AMPK a better substrate for phosphorylation and activation by an upstream kinase (23). Once activated, AMPK phosphorylates targets leading to increased glucose transport, glycolysis, and fatty acid oxidation, as well as decreased ATP consumption (80). Two key phosphorylation targets are the GLUT4 Enhancer Factor (GEF) and Acetyl-CoA Carboxylase (ACC). Phosphorylation of GEF by AMPK results in an increase in GLUT4 expression and, eventually, increased GLUT4 protein accumulation (25, 85), while phosphorylation of ACC inactivates it and causes a decrease in malonyl-CoA levels (33, 82). Malonyl-CoA inhibits carnitine palmitoyltransferase (CPT), which catalyzes a rate-limiting step of fatty-acid β-oxidation (81). The AMPK-caused decrease in malonyl-CoA allows for an increase in CPT activity, thus increasing β-oxidation during exercise. Loss of AMPK

in the skeletal muscle, through the use of a dominant negative form of AMPK's catalytic α subunit, results in the muscles being more sensitive to, and slower to recover from, fatigue (46), and demonstrates the importance of AMPK during exercise.

Additional changes in the muscle during exercise directly affect glycolytic flux, an area that may be mediated by HIF-1α activity. Glucose uptake by the muscle increases dramatically during exercise (37), which is likely a result of increased glucose transporter 4 (GLUT4) translocation to the cell surface (71). Additionally, glycolytic flux is constant and integral during aerobic and anaerobic exercise, and leads to lactate accumulation during both (35).

As can be expected, mutations that block or inhibit steps in these important metabolic pathways can have dramatic phenotypes. Several myopathies have been characterized that result from a blockage in carbohydrate metabolism, and are collectively referred to as glycogen storage diseases (GSD). Two of these diseases are GSD V, muscle glycogen phosphorylase deficiency, and GSD VII, muscle phosphofructokinase deficiency, also known as McArdle's Disease and PFKD, respectively. Patients with either myopathy have decreased carbohydrate utilization resulting in increased glycogen storage, decreased lactate accumulation during exercise, exercise intolerance, and muscle damage following intense exercise (13). As a result of the decreased carbohydrate metabolism, the myopathic muscles frequently have a compensatory response, resulting in their relying more on phosphocreatine and/or aerobic metabolism for ATP production during exertion (2, 73). Another compensatory response, especially in patients with McArdle's Disease, is the Second Wind phenomenon. In this case, normally exercise intolerant patients perform an initial exercise with difficulty, rest briefly, and can then exercise for a significantly longer period of time with much less discomfort. The cause of this phenomenon is not fully understood, but is likely due to compensation from blood glucose and increased fatty acid oxidation (21).

In addition to maintaining ATP levels, another main challenge of skeletal muscle is resisting fatigue. Muscle fibers, and therefore muscles with differing fiber composition, vary in their resistance to fatigue. Type I fibers, which are slow twitch and highly oxidative, are highly fatigue resistant. On the other hand, fast-twitch type II fibers are more susceptible and fatigue quite rapidly. The mechanisms leading to fatigue sensation are not completely understood yet, but to a large degree, are thought to involve lactate signaling. As exercise continues, serum lactate levels increase, and lactate has been shown to correlate well with fatigue sensation. One classic experiment by Fitts and Holloszy (16) demonstrated that muscle contractile force decreases as lactate levels increase. Additionally, administration of dichloroacetate, an activator of pyruvate dehydrogenase (PDH) through inhibition of pyruvate dehydrogenase kinase, decreases lactate accumulation and increases endurance capacity in untrained subjects (41). However, as this process involves PDH activation, and thus will increase oxidative metabolism, the decreased lactate accumulation may merely be correlative to the increase in endurance rather than causative. Other causes of fatigue may be intracellular changes, such as changes in pH, decreased ATP levels, or a failure to regulate Ca^{2+} release or reuptake (12). Since the HIF-1α mediated increase in glycolysis also results in increased lactate production, modulation of HIF-1α in the muscle may have an impact on endurance and fatigue.

Gene transcription in the skeletal muscle is greatly affected both during exercise and recovery following exercise. Expression of interleukin 6, a cytokine that has been proposed to have a large role in fatigue sensation, is markedly increased during exercise (34). The transcription of several important metabolic genes is affected by exercise. In a study looking at gene expression immediately following a four hour cycling exercise in untrained patients, Pilegaard et al. (53) saw elevated expression of heme oxygenase-1 (HO-1) and pyruvate dehydrogenase kinase 4 (PDK4). During the recovery from exercise, muscles further increased PDK4 expression, and also upregulated hexokinase II (HKII), lipoprotein lipase (LPL), and uncoupling protein 3 (UCP3). In a different study, and of specific relation to HIF-1α, expression of VEGF, and its receptor Flt-1 were seen to be upregulated following exercise in rats (51). Additionally, untrained skeletal muscle has a marked upregulation of HIF-1α, HIF-2α, and EPO mRNA during recovery from exercise (1, 42). These transcriptional changes show a coordinated effort by the muscle to adapt to the stress of exercise and become better suited for endurance activities, and also give further evidence for an important role for HIF-1α function in the muscle.

The role of HIF-1 in untrained muscle and during acute exercise has been studied, although its function is not yet completely understood. As mentioned above, resting untrained skeletal muscle has stable HIF-1α protein, suggesting that HIF-1 has an important role in maintaining homeostasis in the muscle. This hypothesis was strengthened by the findings of Ameln et al. (1), who recently showed that acute exercise leads to increased stabilization of HIF-1α protein, perhaps giving the mechanism for the increase in expression of HIF-1 target genes following exercise. However, these earlier studies still did not elucidate the role HIF-1 plays in the way muscles respond during exercise.

With this question in mind, we sought to determine the exact role of HIF-1 signaling in untrained skeletal muscle utilizing a tissue-specific knockout mouse. By crossing mice with LoxP flanked alleles of HIF-1α (59) with mice expressing the Cre recombinase transgene under the control of the muscle creatine kinase promoter (MCK-Cre mice) (7), we were able generate mice lacking HIF-1 signaling in the skeletal muscle (45). Surprisingly, the skeletal-muscle HIF-1α null mice had normal morphology of their muscles, and isolated stimulation of gastrocnemius muscles and single fibers revealed similar force generation, Ca^{++} release, and fatigue rates in control (WT) and HIF-1α null (HIF-null) muscles. However, during these contractions, HIF-null muscles had to rely more heavily on phosphocreatine for ATP generation, and had difficulty maintaining ATP levels. Additionally, the HIF-null muscle accumulated more early glycolytic metabolites, indicating that loss of HIF-1α impeded glycolytic flux in the muscles. Analysis of muscles from mice following a controlled run confirmed this, as HIF-null muscles failed to upregulate expression of key glycolytic enzymes, and were also unable to maintain enzymatic activity of PFK. Correlating with this, the HIF-null mice accumulated less lactate in their serum during the run.

Surprisingly, these changes added up to an increase in endurance for the HIF-null mice when the mice were subjected to swimming and uphill running tests. Further analysis of the HIF-null muscles revealed that loss of HIF-1α lead to an increase in β-hydroxyacyl-CoA dehydrogenase and citrate synthase, indicating increased aerobic ca-

pacity in these mice and contributing to the increase in endurance. Unfortunately, this was not a win-win situation for the mice, as loss of HIF-1α resulted in increased muscle damage following the endurance test. Additionally, when the mice were forced to run downhill, an eccentric exercise that forced the muscles to rely on glycolytic metabolism (48), the HIF-null mice lost their endurance edge due to their impeded glycolytic flux.

From this study, and earlier results, it can be seen that loss of HIF-1α in the skeletal muscle causes an adaptive response leading to an increased capacity for endurance exercise. It can also be seen that HIF-1α is necessary for the maintenance of optimal glycolytic flux in the skeletal muscle. Finally, given the increased muscle damage seen in the HIF-null muscle, it is tempting to speculate that HIF-1α is essential for proper sensation of fatigue, and preventing injury to the muscle from overexertion.

MUSCULAR RESPONSE TO ENDURANCE TRAINING

The ability of the skeletal muscle to acclimate to repeated exertion is central to its role in the body. This ability to acclimate enables it to become better suited and prepared for exercise, something muscle can achieve rather quickly. Endurance training studies have been carried out extensively in humans as well as animal models to understand how muscles undergo this acclimation to exercise. Two main categories that the changes fall under are morphological changes and enzymatic changes, resulting in a change in the profile of the muscle. The end result of endurance training is that the skeletal muscle has improved delivery and utilization of its available oxygen, leading to enhanced performance and endurance. Given that oxygen is central to these changes, it is very likely that the primary hypoxia responsive factor, HIF-1, has a large role in helping the muscle to acclimate to repeated exercise.

The most significant change seen in the muscle as a result of endurance training is increased endurance. However, there are other markers of improved muscle capability beyond just endurance. Two of the more prominent ones are the respiratory exchange ratio (RER) and VO_2max. A measure of fuel utilization, the RER generally has a downward shift following training, indicating an increase in fatty acid oxidation relative to carbohydrate metabolism. VO_2max is the maximal oxygen consumption achievable by the subject, and is closely linked to aerobic metabolic capacity. Like overall endurance, this parameter also usually increases following endurance training, indicating an increase in oxidative capacity by the subject.

Morphologically, there are two main adaptations a muscle undergoes during endurance training – an increase in capillary density and a shift in fiber type composition. The advantage of increased capillary density is obvious as it allows for increased oxygen and metabolite delivery to the exercising muscle, thus increasing aerobic capacity. Increased capillary density can occur after only six to eight weeks of endurance training; this short of a period has been shown to lead to a 30% increase in capillary density (27). The HIF-1α target, VEGF, is of critical importance here as deletion of VEGF in the muscle following development results in a dramatic drop in muscle capillary density and capillary to fiber ratio (68).

The shift in fiber type composition allows the skeletal muscle to better take advantage of this increase in oxygen delivery, and also contributes to the changes in VO_2max and RER that are seen in trained patients and animals. In addition to the two main categories (type II fast twitch and type I slow twitch), muscle fibers can be classified according to their metabolic preferences. Type I fibers are oxidative, and rely heavily on aerobic metabolism, while type II fibers can be broken into two major categories: type IIA and type IIB. Type IIB fibers are largely glycolytic, while type IIA fibers are largely oxidative despite being fast-twitch. Endurance training has been shown to cause a shift toward slow twitch fibers in humans (17). Additionally, trained muscles have a greater percentage of type IIA fibers versus type IIB, indicating an increase in oxidative capacity (24). This shift toward an oxidative profile enables a trained muscle to take full advantage of the increased capillary density.

In addition to morphological changes, there are numerous metabolic changes in trained muscle relative to untrained muscle. Generally, these changes increase the muscle's ability to rapidly produce ATP during exercise, especially from the beta-oxidation of fatty acids. Improvements in ATP production generally come in the form of upregulated metabolic enzymes and the resulting increased capacity for oxidative phosphorylation. Increased oxidative phosphorylation is a result of elevated mitochondrial density in the muscle, and upregulation of levels of the metabolic enzymes contained therein. In previous studies, endurance training has resulted in an increase of 40% in mitochondrial volume in the skeletal muscle, and significant increases have also been seen in the aerobic metabolic enzymes citrate synthase, β-hydroxyacyl-CoA dehydrogenase, and carnitine palmitoyl transferase (4, 22, 27, 61).

Oxidative phosphorylation is not the only metabolic pathway upregulated as a result of training. Activity of hexokinase, a HIF-1 target, also increases as a result of endurance training, indicating improved carbohydrate metabolism (69). The benefit of this increase for the muscle is two-fold. First, as the initial enzyme in glycolysis, an increase in hexokinase activity will allow for greater flux into glycolysis, allowing for greater pyruvate and ATP production. Secondly, since muscle lacks glucose-6-phosphatase, any glucose that enters the muscle will be phosphorylated by hexokinase and remain in the muscle to either be metabolized immediately or stored as glycogen for later use. An increase in hexokinase will thus help ensure there will be enough carbohydrate fuel for the muscle during exercise. In fact, hexokinase activity can control exercise endurance in a dose-dependent manner; in genetic mouse models, increased hexokinase activity was seen to correlate quite well with increased endurance (19).

A third metabolic consequence of endurance training is an increase in glycogen storage in the muscle. This is not only a result of the increased in hexokinase activity, but also a result of increased glycogen synthase (8), and is another way in which a trained muscle is better prepared for exertion. Additionally, endurance-trained muscle is slower to deplete its glycogen stores than untrained muscles, a change which enables muscles to perform longer since they can spare glycogen for when it is absolutely needed (24).

Although not yet completely understood, the mechanism underlying the acclimation of skeletal muscle to endurance training is coming to light, and some of the key factors regulating the response to endurance training have been identified. Surprisingly,

despite the preponderance of HIF-1α targets following exercise, and the importance of angiogenesis to the training response, much of the research into factors regulating the endurance training response has focused on other genes. Two important transcription factors that have a role in upregulating oxidative metabolism are the nuclear respiratory factors 1 and 2 (NRF-1 and 2). NRF-1 and 2 bind to specific response elements of target genes such as mitochondrial transcription factor A (TFAM), cytochrome c, and succinate dehydrogenase subunit B (60). Highlighting the importance of NRF-1, endurance exercise has been shown to increase NRF-1 protein, and a mouse constitutively over expressing NRF-1 has increased oxidative capacity, as well as increased GLUT4 expression (3). However, the NRF-1 transgenic mouse does not have elevated citrate synthase, cyclooxygenase-IV, or succinate:ubiquinol oxidoreductase, indicating that NRF-1 by itself is not sufficient to cause the training-induced changes. Very little research has been done on a connection between HIF-1 and NRF-1, although the two have parallel expression patterns in postnatal hearts (50).

Members of the peroxisome proliferator-activated receptor (PPAR) family have also has been hypothesized to have a role in the muscular response to training. One of them, PPARα, has been shown to upregulate mitochondrial genes in charge of fatty acid oxidation, leading to increased oxidation (20, 72). The primary member of the PPAR family in the skeletal muscle is PPARδ, which has been shown to have an important role in determining muscle oxidative capacity. In work with a PPARδ transgenic mouse, Wang et al. (78) showed that overexpression of PPARδ in the skeletal muscle results in a mouse with a greater proportion of type I oxidative fibers, leading to increased mitochondrial content, resistance to obesity, and dramatically increased endurance. Intriguingly, hypoxia has been shown to down-regulate PPARα, and this down-regulation appears to be HIF-1 dependent (49). It is not currently known if this down-regulation extends to PPARδ as well, but the HIF-1 regulated gene DEC1/Stra13 has been shown to inhibit PPARγ-2 (84). These findings make it interesting to speculate as to whether HIF-1α has a similar interplay with other members of the PPAR family, in particularly PPARδ.

Another gene that has been shown to possibly have a role regulating the muscle response to endurance training is PPARγ coactivator-1α (PGC-1α), which stimulates the expression of NRF-1 and NRF-2, among other genes (83). In the same study, Wu, et al. also saw that PGC-1α binds with NRF-1 and coactivates it at the TFAM promoter, leading to increased mitochondrial biogenesis. In the skeletal muscle, PGC-1α is normally expressed in type I fibers, and constitutive expression of PGC-1α in the muscle at normal physiological levels results in a transition of type II fibers to being more like type I fibers. This results in the fibers becoming more fatigue resistant in isolated stimulation assessments (40).

These three families of genes, the NRFs, PPARs, and PGCs, all have the potential to regulate the changes seen in the muscle. They all increase oxidative capacity and improve muscle performance. Interestingly, not much research has been done on any connections between them and HIF-1, even though the demands of exercise, which induce HIF-1α, are also what lead to their activation, either at the protein level or through transcription (47, 57, 70).

In part because of the repeated oxygen stress placed on skeletal muscle during ex-

ercise, a role for HIF-1α in the muscular response to exercise and training has been proposed (18, 26). Some of the responses seen from muscle during training further corroborate this hypothesis. As hexokinase II and VEGF are two prominent HIF-1α targets, and since an increase in hexokinase and angiogenesis are two common changes following training, a role for HIF-1α can be proposed. Additionally, training under ischemic conditions results in greater citrate synthase activity than exercise with normal blood flow (15, 32). Finally, as mentioned before, transcription of several HIF-1α targets is increased in the muscle following exercise, and transcription of HIF-1α itself is upregulated following repeated hypoxic exercise (74). Thus it can be hypothesized that HIF-1α has a role in the muscular training response to exercise, although no research had directly addressed this prior to our work.

In order to address the role of HIF-1α in skeletal muscle during endurance training, we subjected WT and HIF-null mice to a training protocol. Surprisingly, both genotypes responded equally well to endurance training. Analysis of muscles from mice following training revealed that WT mice were able to "catch up" to HIF-null mice in the areas in which loss of HIF-1α had caused an adaptive response in the muscle. These areas included aerobic metabolism, mitochondrial DNA content, and capillary to fiber ratio. The adaptive response in these parameters in the HIF-null mice was sufficient to endure the training stimulus, and thus no further changes were seen in these parameters. Also consistent with trained muscle, AMPK activation was increased in resting HIF-null muscle, indicating that AMPK signaling has a role in the adaptive response seen in the HIF-null muscles. Hexokinase activity increased in trained muscles of both genotypes, indicating that hexokinase made a strong contribution to the increases in endurance seen, consistent with previous studies (19, 69).

These results, and our work with untrained skeletal muscle, contrast starkly with the hypothesis that HIF-1α plays an integral role in the muscular response to endurance training. Thus, it appears that removing HIF-1 signaling has predisposed the skeletal muscle for endurance training, leading to the speculation that one aspect of endurance training may, in fact, be to remove HIF-1 signaling. Several lines of evidence support this hypothesis. Recent studies have shown that HIF-1α has a suppressive effect on oxidative metabolism; Dahia et al. (11) have shown reduced succinate dehydrogenase subunit B protein levels in response to constitutive HIF-1α activation, and two studies have demonstrated that HIF-1α upregulates pyruvate dehydrogenase kinase I, an inhibitor of pyruvate dehydrogenase and oxidative metabolism (36, 52). Our results corroborate this, as cultured myoblasts lacking HIF-1α have reduced PDK1 protein and increased oxygen consumption in response to hypoxia. Additionally, resting HIF-null skeletal muscle has reduced PDK1 mRNA, something that WT muscle achieves following endurance training.

Thus it is now apparent that HIF-1α signaling actually is inhibitory to endurance training. In keeping with the revised hypothesis that endurance training has a result of removing HIF-1α signaling, Lundby et al. (43) recently demonstrated that induction of HIF-1α mRNA is significantly reduced in trained muscle from human subjects relative to untrained muscle following exercise. While HIF-1α is important for optimal muscle function during acute exercise, it is non-essential for, and likely inhibitory of, endurance training.

REFERENCES

1. Ameln H, Gustafsson T, Sundberg CJ, Okamoto K, Jansson E, Poellinger L, and Makino Y. Physiological activation of hypoxia inducible factor-1 in human skeletal muscle. *Faseb J* 19: 1009-1011, 2005.
2. Argov Z, Bank WJ, Maris J, Leigh JS, Jr., and Chance B. Muscle energy metabolism in human phosphofructokinase deficiency as recorded by 31P nuclear magnetic resonance spectroscopy. *Ann Neurol* 22: 46-51, 1987.
3. Baar K, Song Z, Semenkovich CF, Jones TE, Han DH, Nolte LA, Ojuka EO, Chen M, and Holloszy JO. Skeletal muscle overexpression of nuclear respiratory factor 1 increases glucose transport capacity. *Faseb J* 17: 1666-1673, 2003.
4. Berthon PM, Howlett RA, Heigenhauser GJ, and Spriet LL. Human skeletal muscle carnitine palmitoyltransferase I activity determined in isolated intact mitochondria. *J Appl Physiol* 85: 148-153, 1998.
5. Brooks GA. Mammalian fuel utilization during sustained exercise. *Comp Biochem Physiol B Biochem Mol Biol* 120: 89-107, 1998.
6. Bruick RK, and McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 294: 1337-1340, 2001.
7. Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, and Kahn CR. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 2: 559-569, 1998.
8. Christ-Roberts CY, Pratipanawatr T, Pratipanawatr W, Berria R, Belfort R, Kashyap S, and Mandarino LJ. Exercise training increases glycogen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects. *Metabolism* 53: 1233-1242, 2004.
9. Cockman ME, Masson N, Mole DR, Jaakkola P, Chang GW, Clifford SC, Maher ER, Pugh CW, Ratcliffe PJ, and Maxwell PH. Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. *J Biol Chem* 275: 25733-25741, 2000.
10. Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, Firestein GS, Gerber HP, Ferrara N, and Johnson RS. HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell* 112: 645-657, 2003.
11. Dahia PL, Ross KN, Wright ME, Hayashida CY, Santagata S, Barontini M, Kung AL, Sanso G, Powers JF, Tischler AS, Hodin R, Heitritter S, Moore F, Dluhy R, Sosa JA, Ocal IT, Benn DE, Marsh DJ, Robinson BG, Schneider K, Garber J, Arum SM, Korbonits M, Grossman A, Pigny P, Toledo SP, Nose V, Li C, and Stiles CD. A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. *PLoS genetics* 1: 72-80, 2005.
12. Dalakas MC, Mock V, and Hawkins MJ. Fatigue: definitions, mechanisms, and paradigms for study. *Semin Oncol* 25: 48-53, 1998.
13. DiMauro S, Bresolin N, and Hays AP. Disorders of glycogen metabolism of muscle. *CRC Crit Rev Clin Neurobiol* 1: 83-116, 1984.
14. Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, and Ratcliffe PJ. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 107: 43-54, 2001.

15. Esbjornsson M, Jansson E, Sundberg CJ, Sylven C, Eiken O, Nygren A, and Kaijser L. Muscle fibre types and enzyme activities after training with local leg ischaemia in man. *Acta Physiol Scand* 148: 233-241, 1993.
16. Fitts RH, and Holloszy JO. Lactate and contractile force in frog muscle during development of fatigue and recovery. *Am J Physiol* 231: 430-433, 1976.
17. Fluck M, and Hoppeler H. Molecular basis of skeletal muscle plasticity--from gene to form and function. *Rev Physiol Biochem Pharmacol* 146: 159-216, 2003.
18. Freyssenet DG. Energy sensing and regulation of gene expression in skeletal muscle. *J Appl Physiol* 2006.
19. Fueger PT, Shearer J, Krueger TM, Posey KA, Bracy DP, Heikkinen S, Laakso M, Rottman JN, and Wasserman DH. Hexokinase II protein content is a determinant of exercise endurance capacity in the mouse. *The Journal of physiology* 566: 533-541, 2005.
20. Gulick T, Cresci S, Caira T, Moore DD, and Kelly DP. The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc Natl Acad Sci U S A* 91: 11012-11016, 1994.
21. Haller RG, and Vissing J. Spontaneous "second wind" and glucose-induced second "second wind" in McArdle disease: oxidative mechanisms. *Arch Neurol* 59: 1395-1402, 2002.
22. Harms SJ, and Hickson RC. Skeletal muscle mitochondria and myoglobin, endurance, and intensity of training. *J Appl Physiol* 54: 798-802, 1983.
23. Hawley SA, Selbert MA, Goldstein EG, Edelman AM, Carling D, and Hardie DG. 5'-AMP activates the AMP-activated protein kinase cascade, and Ca2+/calmodulin activates the calmodulin-dependent protein kinase I cascade, via three independent mechanisms. *J Biol Chem* 270: 27186-27191, 1995.
24. Holloszy JO, and Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol* 56: 831-838, 1984.
25. Holmes BF, Sparling DP, Olson AL, Winder WW, and Dohm GL. Regulation of muscle GLUT4 enhancer factor and myocyte enhancer factor 2 by AMP-activated protein kinase. *Am J Physiol Endocrinol Metab* 289: E1071-1076, 2005.
26. Hoppeler H, and Fluck M. Normal mammalian skeletal muscle and its phenotypic plasticity. *J Exp Biol* 205: 2143-2152, 2002.
27. Hoppeler H, Howald H, Conley K, Lindstedt SL, Claassen H, Vock P, and Weibel ER. Endurance training in humans: aerobic capacity and structure of skeletal muscle. *J Appl Physiol* 59: 320-327, 1985.
28. Huang LE, Gu J, Schau M, and Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. *Proc Natl Acad Sci U S A* 95: 7987-7992, 1998.
29. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, and Kaelin WG, Jr. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science* 292: 464-468, 2001.
30. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, and Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science* 292: 468-472, 2001.
31. Jewell UR, Kvietikova I, Scheid A, Bauer C, Wenger RH, and Gassmann M. Induction of HIF-1alpha in response to hypoxia is instantaneous. *Faseb J* 15: 1312-1314, 2001.
32. Kaijser L, Sundberg CJ, Eiken O, Nygren A, Esbjornsson M, Sylven C, and Jansson E. Muscle oxidative capacity and work performance after training under local leg ischemia. *J Appl Physiol* 69: 785-787, 1990.

33. Kaushik VK, Young ME, Dean DJ, Kurowski TG, Saha AK, and Ruderman NB. Regulation of fatty acid oxidation and glucose metabolism in rat soleus muscle: effects of AICAR. *Am J Physiol Endocrinol Metab* 281: E335-340, 2001.
34. Keller C, Steensberg A, Pilegaard H, Osada T, Saltin B, Pedersen BK, and Neufer PD. Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *Faseb J* 15: 2748-2750, 2001.
35. Kemper WF, Lindstedt SL, Hartzler LK, Hicks JW, and Conley KE. Shaking up glycolysis: Sustained, high lactate flux during aerobic rattling. *Proc Natl Acad Sci U S A* 98: 723-728, 2001.
36. Kim JW, Tchernyshyov I, Semenza GL, and Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell metabolism* 3: 177-185, 2006.
37. Kjaer M, Kiens B, Hargreaves M, and Richter EA. Influence of active muscle mass on glucose homeostasis during exercise in humans. *J Appl Physiol* 71: 552-557, 1991.
38. Kondo K, and Kaelin WG, Jr. The von Hippel-Lindau tumor suppressor gene. *Exp Cell Res* 264: 117-125, 2001.
39. Liao D, Corle C, Seagroves TN, and Johnson RS. Hypoxia-inducible factor-1alpha is a key regulator of metastasis in a transgenic model of cancer initiation and progression. *Cancer Res* 67: 563-572, 2007.
40. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, and Spiegelman BM. Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature* 418: 797-801, 2002.
41. Ludvik B, Mayer G, Stifter S, Putz D, Barnas U, and Graf H. Effects of dichloroacetate on exercise performance in healthy volunteers. *Pflugers Arch* 423: 251-254, 1993.
42. Lundby C, Gassmann M, and Pilegaard H. Regular endurance training reduces the exercise induced HIF-1alpha and HIF-2alpha mRNA expression in human skeletal muscle in normoxic conditions. *Eur J Appl Physiol* 1-7, 2005.
43. Lundby C, Gassmann M, and Pilegaard H. Regular endurance training reduces the exercise induced HIF-1alpha and HIF-2alpha mRNA expression in human skeletal muscle in normoxic conditions. *European journal of applied physiology* 96: 363-369, 2006.
44. Madan A, and Curtin PT. A 24-base-pair sequence 3' to the human erythropoietin gene contains a hypoxia-responsive transcriptional enhancer. *Proc Natl Acad Sci U S A* 90: 3928-3932, 1993.
45. Mason SD, Howlett RA, Kim MJ, Olfert IM, Hogan MC, McNulty W, Hickey RP, Wagner PD, Kahn CR, Giordano FJ, and Johnson RS. Loss of skeletal muscle HIF-1alpha results in altered exercise endurance. *PLoS Biol* 2: e288, 2004.
46. Mu J, Barton ER, and Birnbaum MJ. Selective suppression of AMP-activated protein kinase in skeletal muscle: update on 'lazy mice'. *Biochem Soc Trans* 31: 236-241, 2003.
47. Murakami T, Shimomura Y, Yoshimura A, Sokabe M, and Fujitsuka N. Induction of nuclear respiratory factor-1 expression by an acute bout of exercise in rat muscle. *Biochim Biophys Acta* 1381: 113-122, 1998.
48. Nardone A, and Schieppati M. Shift of activity from slow to fast muscle during voluntary lengthening contractions of the triceps surae muscles in humans. *The Journal of physiology* 395: 363-381, 1988.
49. Narravula S, and Colgan SP. Hypoxia-inducible factor 1-mediated inhibition of peroxisome proliferator-activated receptor alpha expression during hypoxia. *J*

Immunol 166: 7543-7548, 2001.
50. Nau PN, Van Natta T, Ralphe JC, Teneyck CJ, Bedell KA, Caldarone CA, Segar JL, and Scholz TD. Metabolic adaptation of the fetal and postnatal ovine heart: regulatory role of hypoxia-inducible factors and nuclear respiratory factor-1. *Pediatr Res* 52: 269-278, 2002.
51. Olfert IM, Breen EC, Mathieu-Costello O, and Wagner PD. Chronic hypoxia attenuates resting and exercise-induced VEGF, flt-1, and flk-1 mRNA levels in skeletal muscle. *J Appl Physiol* 90: 1532-1538, 2001.
52. Papandreou I, Cairns RA, Fontana L, Lim AL, and Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell metabolism* 3: 187-197, 2006.
53. Pilegaard H, Ordway GA, Saltin B, and Neufer PD. Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. *Am J Physiol Endocrinol Metab* 279: E806-814, 2000.
54. Pisani DF, and Dechesne CA. Skeletal muscle HIF-1alpha expression is dependent on muscle fiber type. *J Gen Physiol* 126: 173-178, 2005.
55. Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, and Wagner PD. Myoglobin O2 desaturation during exercise. Evidence of limited O2 transport. *J Clin Invest* 96: 1916-1926, 1995.
56. Rowell LB. Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev* 54: 75-159, 1974.
57. Russell AP, Hesselink MK, Lo SK, and Schrauwen P. Regulation of metabolic transcriptional co-activators and transcription factors with acute exercise. *Faseb J* 19: 986-988, 2005.
58. Ryan HE, Lo J, and Johnson RS. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *Embo J* 17: 3005-3015, 1998.
59. Ryan HE, Poloni M, McNulty W, Elson D, Gassmann M, Arbeit JM, and Johnson RS. Hypoxia-inducible factor-1alpha is a positive factor in solid tumor growth. *Cancer Res* 60: 4010-4015, 2000.
60. Scarpulla RC. Nuclear activators and coactivators in mammalian mitochondrial biogenesis. *Biochim Biophys Acta* 1576: 1-14, 2002.
61. Schantz P, Henriksson J, and Jansson E. Adaptation of human skeletal muscle to endurance training of long duration. *Clin Physiol* 3: 141-151, 1983.
62. Schipani E, Ryan HE, Didrickson S, Kobayashi T, Knight M, and Johnson RS. Hypoxia in cartilage: HIF-1alpha is essential for chondrocyte growth arrest and survival. *Genes Dev* 15: 2865-2876, 2001.
63. Seagroves TN, Ryan HE, Lu H, Wouters BG, Knapp M, Thibault P, Laderoute K, and Johnson RS. Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. *Mol Cell Biol* 21: 3436-3444, 2001.
64. Semenza G. Signal transduction to hypoxia-inducible factor 1. *Biochem Pharmacol* 64: 993-998, 2002.
65. Semenza GL. HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell* 107: 1-3, 2001.
66. Silva JL, Giannocco G, Furuya DT, Lima GA, Moraes PA, Nachef S, Bordin S, Britto LR, Nunes MT, and Machado UF. NF-kappaB, MEF2A, MEF2D and HIF1-a involvement on insulin- and contraction-induced regulation of GLUT4 gene expression in soleus muscle. *Mol Cell Endocrinol* 240: 82-93, 2005.
67. Stroka DM, Burkhardt T, Desbaillets I, Wenger RH, Neil DA, Bauer C, Gassmann M, and Candinas D. HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. *Faseb J* 15: 2445-2453, 2001.

68. Tang K, Breen EC, Gerber HP, Ferrara NM, and Wagner PD. Capillary regression in vascular endothelial growth factor-deficient skeletal muscle. *Physiol Genomics* 18: 63-69, 2004.
69. Taylor EB, Lamb JD, Hurst RW, Chesser DG, Ellingson WJ, Greenwood LJ, Porter BB, Herway ST, and Winder WW. Endurance training increases skeletal muscle LKB1 and PGC-1alpha protein abundance: effects of time and intensity. *American journal of physiology* 289: E960-968, 2005.
70. Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T, and Tabata I. Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. *Biochem Biophys Res Commun* 296: 350-354, 2002.
71. Thorell A, Hirshman MF, Nygren J, Jorfeldt L, Wojtaszewski JF, Dufresne SD, Horton ES, Ljungqvist O, and Goodyear LJ. Exercise and insulin cause GLUT-4 translocation in human skeletal muscle. *Am J Physiol* 277: E733-741, 1999.
72. Vega RB, Huss JM, and Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol* 20: 1868-1876, 2000.
73. Vissing J, Galbo H, and Haller RG. Paradoxically enhanced glucose production during exercise in humans with blocked glycolysis caused by muscle phosphofructokinase deficiency. *Neurology* 47: 766-771, 1996.
74. Vogt M, Puntschart A, Geiser J, Zuleger C, Billeter R, and Hoppeler H. Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. *J Appl Physiol* 91: 173-182, 2001.
75. Wang GL, Jiang BH, Rue EA, and Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc Natl Acad Sci U S A* 92: 5510-5514, 1995.
76. Wang GL, and Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc Natl Acad Sci U S A* 90: 4304-4308, 1993.
77. Wang GL, and Semenza GL. Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* 270: 1230-1237, 1995.
78. Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, Bayuga-Ocampo CR, Ham J, Kang H, and Evans RM. Regulation of muscle fiber type and running endurance by PPARdelta. *PLoS Biol* 2: e294, 2004.
79. Wiesener MS, Jurgensen JS, Rosenberger C, Scholze CK, Horstrup JH, Warnecke C, Mandriota S, Bechmann I, Frei UA, Pugh CW, Ratcliffe PJ, Bachmann S, Maxwell PH, and Eckardt KU. Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. *Faseb J* 17: 271-273, 2003.
80. Winder WW. Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. *J Appl Physiol* 91: 1017-1028, 2001.
81. Winder WW, Arogyasami J, Barton RJ, Elayan IM, and Vehrs PR. Muscle malonyl-CoA decreases during exercise. *J Appl Physiol* 67: 2230-2233, 1989.
82. Winder WW, Wilson HA, Hardie DG, Rasmussen BB, Hutber CA, Call GB, Clayton RD, Conley LM, Yoon S, and Zhou B. Phosphorylation of rat muscle acetyl-CoA carboxylase by AMP-activated protein kinase and protein kinase A. *J Appl Physiol* 82: 219-225, 1997.
83. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, and Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98: 115-124, 1999.
84. Yun Z, Maecker HL, Johnson RS, and Giaccia AJ. Inhibition of PPAR gamma 2 gene

expression by the HIF-1-regulated gene DEC1/Stra13: a mechanism for regulation of adipogenesis by hypoxia. *Dev Cell* 2: 331-341, 2002.

85. Zheng D, MacLean PS, Pohnert SC, Knight JB, Olson AL, Winder WW, and Dohm GL. Regulation of muscle GLUT-4 transcription by AMP-activated protein kinase. *J Appl Physiol* 91: 1073-1083, 2001.