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...
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