Qomolangma: A Novel and Effective Weight Loss Method

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This is to acknowledge that Dr. Palmer has not disclosed any financial interests or other relationships with commercial concerns related directly to this program. Dr. Palmer will not be discussing off-label uses in his presentation.

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Purpose: Given the increasing prevalence of obesity worldwide and its associated adverse effects on quality of life and average life expectancy, there is a need for more effective weight loss strategies. While sojourns to altitude may not be a feasible strategy to lose weight for the vast majority of obese subjects, understanding the mechanisms by which altitude exposure causes weight loss in the face of reduced appetite and increased energy expenditure can lead to new tools for treating obesity in the general population.

Educational Objectives:

1. Hypobaric hypoxia leads to up regulation of hypoxia inducible factor (HIF).
2. Activation of HIF leads to alterations in metabolism which can importantly contribute to the various mechanisms accounting for weight loss at altitude to include increased basal metabolic rate and suppression of appetite.
3. There are genetic and gender differences which influence the degree to which HIF is activated under conditions of hypoxia. Such differences may be important in the degree to which body weight and exercise performance changes upon exposure to altitude.

Introduction

Weight gain occurs when energy intake from food exceeds energy expenditure required for everyday living. By contrast, restricting caloric intake to a value below that needed for daily energy requirements will result in weight loss. A secondary or combinatorial method of weight reduction is to increase physical activity or the metabolic rate/energy expenditure by an amount greater than caloric intake. Many patients find it extremely difficult to initiate and maintain the behavioral patterns of diet and exercise required to achieve weight reduction. Currently, the most efficacious and sustainable weight loss regime is bariatric surgery. While bariatric procedures are effective weight loss methods, they are invasive and can be associated with morbidity. Currently, pharmacologic interventions are limited by availability of drugs, overall efficacy, and side effects. Given the increasing prevalence of obesity worldwide and its associated adverse effects on quality of life and average life expectancy, there is a need for more effective weight loss strategies.

The drive and requirement to eat food is a major barrier limiting the success of any weight loss initiative. Hunger is invariably associated with caloric restriction. In addition, weight loss in otherwise normal subjects is accompanied by a decrease in energy expenditure. Reductions in energy expenditure mirror the decline in body weight following any given reduction in caloric intake adding to the difficulty obese subjects face when initiating or maintaining a diet. The most effective weight loss strategy is to suppress appetite and at the same time maintain or even increase energy expenditure. Such a situation occurs in disease states such as chronic obstructive pulmonary disease and cancer. In these conditions a persistent loss of appetite plays a major role in the progressive weight loss often observed in these patients. In addition these diseases are characterized by an increase in energy expenditure that persists in the face of weight reduction further contributing to progressive weight loss.

One situation in which weight loss is accompanied by appetite suppression and increased energy expenditure is when otherwise healthy subjects are taken to altitude. In a recent study 20 male obese subjects were taken by train to an environmentally controlled research station at an altitude of 2650 meters (m) (1). For a period of seven days the subjects were allowed to eat and drink without restriction and physical activity was limited to slow walks throughout the station. Body weight at the end of seven days decreased from 105.1 kg to 103.6 kg. Caloric intake decreased on average by 734 kcal/day corresponding to 5138 kcal/week. Basal metabolic rate was increased at the end of the seven day period in comparison to baseline values. Interestingly sustained body weight reductions were observed in the participants after returning to low altitude (530 m) 4 weeks later.

While the cause of weight loss in chronic obstructive pulmonary disease and cancer is complex and multifactorial, a feature these diseases share in common with normal subjects exposed to altitude is hypoxia. At altitude hypoxia is secondary to the reduction in barometric pressure causing a decrease in the inspired partial pressure of oxygen whereas hypoxia is due to parenchymal lung injury in chronic obstructive pulmonary disease. Hypoxia in cancer patients is more localized and results from tumor growth outstripping its vascular supply. Of note, there is an ongoing series of investigations probing if obesity generates hypoxic adipose tissue analogous to what occurs in cancer, as indicated by the reductions in vascular supply into obese adipose tissue (2).

This review will examine the physiologic response to hypoxia and focus on those mechanisms which may contribute to weight loss in these otherwise disparate conditions. Several points will be emphasized. First, hypobaric hypoxia leads to up regulation of hypoxia

inducible factor (HIF). Second, activation of HIF leads to alterations in metabolism which can importantly contribute to the various mechanisms accounting for weight loss at altitude to include increased basal metabolic rate and suppression of appetite. Third, there are genetic and gender differences which influence the degree to which HIF is activated under conditions of hypoxia. Such differences may be important in the degree to which body weight and exercise performance changes upon exposure to altitude. While sojourns to altitude may not be a feasible strategy to lose weight for the vast majority of obese subjects, understanding the mechanisms by which altitude exposure causes weight loss in the face of reduced appetite and increased energy expenditure can lead to new tools for treating obesity in the general population.

Hypoxia Inducible Factor (HIF)

Hypobaric hypoxia is thought to be a major factor in the loss of weight and appetite characteristic of climbers on high altitude climbing expeditions. Weight loss under condition of chronic hypoxia is accompanied by adaptive changes in cardiovascular function, energy metabolism, and hormone production designed to maintain oxygen delivery to tissues (3). Adaptive changes also occur at the cellular level through transcriptional activation of various genes designed to reduce oxygen consumption and limit the damaging effects of oxygen lack. An essential mediator of this cellular response is the HIF family of transcription factors.

HIF is a DNA-binding transcription factor which in association with specific nuclear cofactors transactivates a variety of genes triggering an adaptive response aimed to optimize the utilization of available oxygen under conditions of hypoxia (4,5). The three members of the family (HIF-1, HIF-2 and HIF-3) are heterodimers consisting of an oxygen-sensitive α-subunit and a constitutively expressed β-subunit. In the presence of oxygen, HIFα is extremely unstable due to an oxygen-dependent hydroxylation which targets it for proteosomal degradation. This hydroxylation is mediated by three prolyl hydroxylases (PHD1-3) and requires oxygen as well as Fe^{2+}, 2-oxoglutarate, and ascorbate for their catalytic activity (6). Prolyl-hydroxylated HIFα is bound by the von Hippel-Lindau (VHL) tumor suppressor protein and subsequently ubiquitylated by the Elongin C-Elongin B-Cullin 2-E3-ubiquitin-lagase complex marking HIFα for proteosomal degradation. The requirement of oxygen for the catalytic activity of PHD1-3 allows for HIFα to escape recognition by VHL under conditions of hypoxia. In this setting HIFα is stabilized and accumulates in the nucleus where it dimerizes with HIFβ and subsequently binds to the hypoxia response element in target genes.

Activation of these genes give rise to a myriad of effects designed to promote survival in low-oxygen conditions. These include increases in red cell mass brought about by stimulation of erythropoietin production and promotion of angiogenesis through stimulation of vascular endothelial growth factor (VEGF). HIF-induced gene activation also causes a coordinated shift in metabolism from oxidative phosphorylation to a less oxygen requiring production of ATP via the glycolytic pathway (7). A critical step in this shift is HIF-mediated activation of pyruvate dehydrogenase kinase-1 (PDK-1). This enzyme inactivates pyruvate kinase which is the mitochondrial enzyme responsible for converting pyruvate to acetyl-CoA. In combination with activation of lactate dehydrogenase A (LDHA) which converts pyruvate to lactate, there is less delivery of acetyl-CoA into the Krebs cycle and therefore a reduction of flavin adenine dinucleotide (FADH2) and nicotinamide adenine dinucleotide (NADH) delivered to the electron transport chain. This shift away from mitochondrial respiration serves to reduce the production of potentially harmful reactive oxygen species.

Under normal circumstances components of the electron transport chain generate reactive oxygen species but at low levels (8,9). This production increases in the setting of hypoxia due to electrons being delivered to oxygen prior to the reduction of oxygen to water at cytochrome c oxidase, a phenomena referred to as electron leak. The accumulation of reactive oxygen species into the cytosol specifically generated by mitochondrial complex III has been shown to provide an additional trigger leading to the stabilization of HIFα (10). HIF also increases the efficiency of remaining aerobic metabolism by modulating the makeup of complex IV (the last complex in the electron transport chain). HIF induces a switch in subunits from COX4-1 to COX4-2 which is more efficient at facilitating the transfer of electrons to oxygen (11).

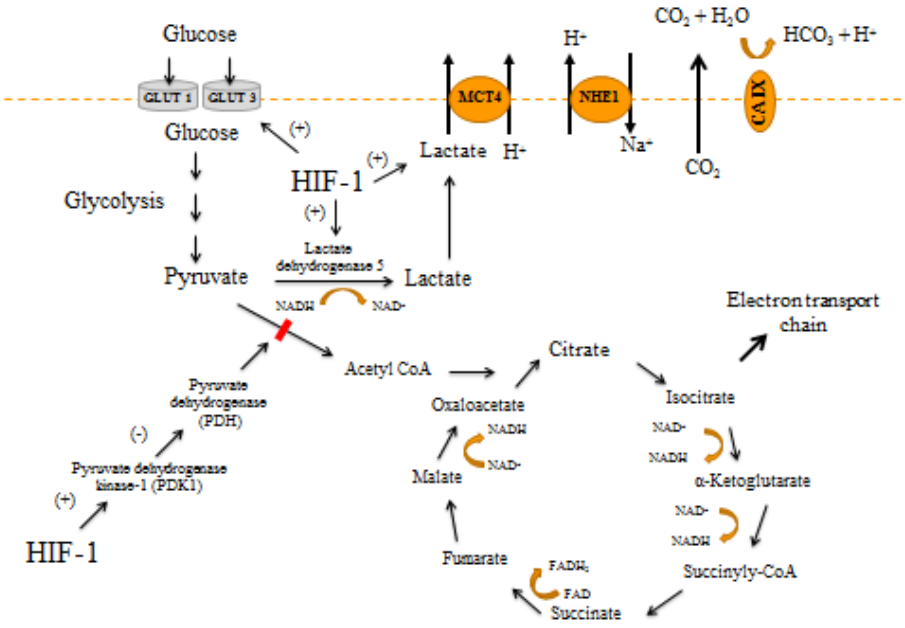

Figure 1. HIF shifts metabolism away from oxidative phosphorylation towards anaerobic metabolism

The switch to glycolysis and lactate production in the hypoxic cell requires an adequate supply of glucose. HIF up regulates the expression of GLUT1 and GLUT3 on the cell membrane so as to facilitate glucose entry into the cell (12). Some of this glucose is directed towards glycogen synthesis under the dictates of HIF thereby ensuring an additional source of substrate for ongoing glycolysis (13). HIF also up regulates monocarboxylate transporter 4 which is a proton-lactate symporter. This transporter creates an avenue for lactate exit from the cell. The cotransport of the proton contributes to the maintenance of intracellular pH. Cell pH is also defended by HIF-induced expression of carbonic anhydrase-9 and increased expression of the Na^+/H^+ antiporter, NHE1 (14).

Possible role of HIF in the increase BMR at Altitude

The change in metabolism favoring increased cellular glucose uptake is consistent with findings reported in normal subjects taken to altitude (15-17). Glucose kinetic studies using radiolabeled tracers show high altitude is associated with increased rates of glucose appearance, disappearance, and oxidation both at rest and exercise compared to sea level. In these studies the resting plasma glucose concentration decreases upon altitude exposure. When energy intake is increased so as to maintain body weight this increased dependency on glucose oxidation persists and occurs at the expense of decreased lipid oxidation. Since metabolism of glucose via glycolysis requires less oxygen as compared to oxidative phosphorylation this shift in metabolism represents an adaptive response to limited oxygen availability.

The redirection of glucose metabolism toward production of lactate may be of importance in supporting the shift to glycolytic metabolism at high altitude by way of the Cori cycle. Cell lactate is exported and taken up by the liver and kidney in a more efficient manner where it can then serve as a substrate for increased gluconeogenesis. In this regard, both hepatic lactate uptake and glucose output increase several fold when normal subjects performing submaximal exercise are switched from a normoxic to a hypoxic breathing mixture (18). In addition, hypoxia increases glucose output in hepatocytes in association with transcriptional activation of phosphoenolpyruvate carboxykinase (PEPCK), the rate limiting enzyme for gluconeogenesis (19). HIF directly binds to the promoter region of PEPCK providing a molecular mechanism by which hypoxia can stimulate hepatic gluconeogenesis. Gluconeogenesis in the kidney is also markedly increased in rats chronically exposed to a simulated altitude of 18000 feet (20). Taken together, increased gluconeogenesis under conditions of hypoxia may serve as a mechanism to minimize accumulation of lactate in the setting of increased cellular production and provide adequate amounts of glucose substrate in order to support the switch in fuel preference.

The increase in energy expenditure which persists despite weight reduction in hypobaric hypoxia may in part be related to the shift in metabolism away from oxidative phosphorylation. Such a situation is well recognized to occur in cancer (21). The rapid expansion of proliferating cells in tumors can outstrip their vascular supply leading to regions of hypoxia. In these zones HIF is stabilized and results in a shift to glycolysis as the primary means for ATP synthesis. This phenomena known as the Warburg effect leads to increased lactic acid production and glucose uptake in cancer cells which are known indicators of tumor aggressiveness and poor patient prognosis. This metabolic shift is energy inefficient since only 2 ATP's are formed for each mol of glucose as compared to 38 ATP's formed when glucose is metabolized via oxidative phosphorylation. It is estimated tumors require a 40 fold greater amount of glucose to provide the energy for growth because of this shift in metabolism (22). In a manner similar to what was described previously, lactate produced in tumors is delivered to the liver where it is resynthesized into glucose. Hepatic gluconeogenesis contributes to the energy wasting in that 6 ATP's are consumed in order to generate 1 mol of glucose from 2 mols of lactic acid. The increase in Cori Cycle activity has been estimated to account for 300 kcal/day of additional energy loss in cancer patients (23). Cori cycle activity and glucose production and turnover rates are significantly greater in cancer patients with progressive weight loss in comparison to those whose weight is stable (24,25).

The increase in basal metabolic rate (BMR) upon exposure to altitude may be a manifestation of this energy wasting cycle. The BMR typically increases in lowlanders by 6-27% over the first several days after arrival at high altitude and then tends to slowly decline with acclimatization. During prolonged stays up to 5000 meters the BMR tends to return towards normal values but remains persistently increased at more extreme elevations (26,27). The initial increase in BMR is directly related to the altitude attained. At 3650 m BMR increases by 6% whereas increases of 10% and 27% occur at 3800 and 4300 m respectively (28-30). In some reports the BMR declines back to normal after several weeks whereas others report a sustained elevation. Part of this variability may be due to differences in the magnitude of weight loss. In those reports where BMR returns to sea-level values, loss of metabolically active tissue may be more pronounced accounting for the normalization. However, when energy balance is maintained so as to prevent weight loss, the increase in BMR is persistent. For example, in one study acute altitude exposure to 4300 m increased basal metabolic rate by 27% over that of sea

level and remained elevated by 17% after 3 weeks of acclimatization. In this study weight loss was minimized by a concerted effort to match energy intake to the measured energy requirement.

The maximal increase in BMR soon after arrival to altitude followed by a slow decline towards baseline values with acclimatization mirrors the pattern of HIF activity upon exposure to hypoxia. Upregulation of HIF activity upon acute exposure to hypoxia tends to decline towards a lower steady state value with chronic exposure suggesting the presence of a feedback mechanism. In this regard both PHD2 and PHD3 are HIF-dependent genes and retain some level of activity under conditions of hypoxia (31). In addition HIF-1α has been shown to upregulate the microRNA miR-155 which in turn exerts an inhibitory effect on HIF-1α mRNA translation (32). The presence of an effective negative feedback loop provides a means to limit excessive HIF signaling which could prove detrimental to cells over the long term as well accelerate the degradation of HIF following re-oxygenation.

In mice exposed to a simulated altitude of 4300 m muscle protein levels of HIF-1α increased by 70% over control values but returned to baseline when measured after one week of exposure (33). A similar pattern was seen in the muscle protein level of PDK1. When animals were exercised on a treadmill, blood and muscle lactate levels were significantly greater in mice exposed to 24 hours of hypoxia but returned towards normoxic levels when exercise was repeated at one week of hypoxic exposure. These data suggest chronic hypoxic exposure is associated with a down regulation of HIF and a secondary reduction of PDK1 levels. These changes allow exercising muscle to assume a phenotype more reminiscent of normoxia where pyruvate conversion to acetyl-CoA is preferred and conversion to lactate is reduced.

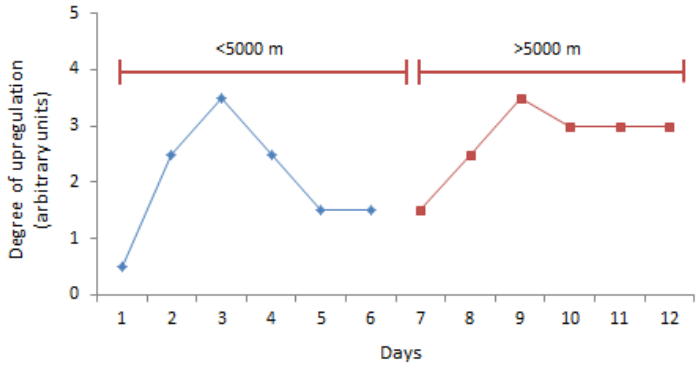

Figure 2. Proposed model of HIF activation in relationship to altitude

Altitude acclimatization has been reported to blunt the accumulation of lactate during periods of exertion despite the persistence of hypoxia (34). Although controversial, this phenomenon has been referred to as the lactate paradox. It is interesting to speculate that in a manner similar to that observed in experimental animals, down regulation of HIF with chronic hypobaric hypoxia may decrease pyruvate to lactate flux and account for this metabolic change.

Changes in HIF activity during acute and chronic hypoxic exposure may also explain the pattern of erythropoietin secretion measured at altitude. Circulating levels of erythropoietin increase rapidly with peak levels occurring 48-72 hours after arrival to altitude. This rise is short lived however as levels return toward baseline values over the subsequent 5-10 days of acclimatization (35,36). With further ascent erythropoietin levels once again increase and depending on the degree of elevation may remain significantly elevated. Using erythropoietin levels as a biomarker of HIF activity, this pattern suggests feedback inhibition of HIF activity can be overridden by more extreme hypobaric hypoxia which accompanies higher altitudes. In this setting, high steady state levels

of HIF activity may lead to untoward effects particularly with regards to the characteristics of weight loss.

Characteristics of Weight Loss with Altitude

At moderate altitude the most significant change in body composition is a loss of body fat accounting for 70% of the weight reduction on a trek to 5400 meters. At more extreme elevations muscle protein catabolism becomes the dominant change as loss of fat accounts for only 27% of any further weight loss (37). These changes underlie the decision to place base camps at altitudes no higher than 5000 meters during high altitude mountaineering expeditions. At this altitude weight loss from a reduction in fat and muscle can be minimized by maintaining adequate dietary intake.

Operation Everest II was a study in which normal subjects were placed in a decompression chamber at sea level and exposed to a progressive lowering of inspired O_2 pressure so as to simulate a 40 day ascent of Mt. Everest (26). In this setting any change in weight or appetite could be more confidently attributed to hypobaric hypoxia since the participants were not subjected to cold, overexertion, or other rigors of climbing high mountains. Body weight was reduced by 7.44 kg over the study period representing an 8.9% decline from the initial body weight (21,22); however in addition to weight loss, total muscle area calculated in 6 subjects from CT scans of the thigh and upper arms was reduced by 13 and 15% respectively.

The muscle wasting which occurs at extreme altitude is accompanied by a substantial decrease in mitochondrial volume density in skeletal muscle (38-40). Skeletal muscle biopsies taken from climbers on Mt Everest show a decrease in mitochondrial volume by up to 30% primarily accounted for by a reduction in the subsarcolemmal subpopulation of mitochondria. The change in mitochondrial volume is accompanied by significant decreases in the activity of enzymes responsible for aerobic oxidative metabolism. Similar effects on muscle and tissue oxidative capacity were found in subjects exposed to simulated altitude in the Operation Everest II project.

Endurance training at sea level leads to adaptions in skeletal muscle mitochondria that favor fatty acid oxidation. Rates of fatty acid oxidation increase to a much greater extent in the subsarcolemmal subpopulation as compared to intermyofibrillar mitochondria (41). The preferential reduction in subsarcolemmal as compared to intermyofibrillar mitochondria at altitude may be a part of the generalized shift away from oxidative phosphorylation to anaerobic metabolism utilizing glucose as the preferred fuel substrate.

The change in skeletal muscle mitochondria at altitude is likely to be mediated by upregulated HIF (42). Peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α and its homolog PGC-1β are transcriptional coactivators abundant in skeletal muscle and stimulate mitochondrial biogenesis (43). HIF leads to a downregulation of PGC-1α potentially accounting for the reduction in mitochondrial volume noted on muscle biopsies taken from climbers at high altitude (44,45). In this regard levels of PGC-1α are decreased by 35% in climbers after extended stay at altitude with ascent beyond 6400 meters (40). PGC-1α also normally induces a remodeling of skeletal muscle fiber composition such that the ratio of more oxidative type I fibers to the glycolytic type IIb fibers is increased. A reduction in this ratio due to HIF-mediated suppression of PGC-1α would fit with the body wide shift towards glycolytic metabolism. HIF has also been shown to induce mitochondrial autophagy by upregulating the pro-apoptotic protein BCL2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3) (46). BNIP3

disrupts the interaction of Beclin-1 with Bcl2 leading to Atg-5 dependent mitochondrial autophagy.

To summarize, HIF controls a series of molecular events designed to maintain energy homeostasis and limit oxidative stress under conditions of hypoxia. First, HIF increases the efficiency of cytochrome c oxidase under conditions of hypoxia by inducing a subunit switch from COX4-1 to COX4-2. Second, HIF activates a series of genes encoding glucose transporters and glycolytic enzymes designed to increase flux from glucose to lactate and simultaneously shunt pyruvate away from the mitochondria by preventing its metabolism to acetyl CoA. Third, HIF suppresses mitochondrial biogenesis and simultaneously induces mitochondrial autophagy.

Decreased Appetite with Altitude

The decrease in appetite and increase in energy expenditure along with the magnitude of weight loss are related to the altitude attained and duration of stay. Anorexia tends to be maximal in the first several days upon arrival when protein and caloric intake can decrease as much as 30 and 40% respectively. Below 5000 meters food intake tends to return towards normal over several days during the process of acclimatization (47). At more extreme altitudes anorexia is more pronounced and becomes persistent.

The Operation Everest III study examined the long term effect of hypobaric hypoxia on appetite using a hypobaric chamber and simulating the ascent of Mount Everest over a 31 day period (48). Weight was reduced by an average of 5 kilograms in the study participants. A reduction in appetite with reduced energy intake was the primary factor responsible for the reduction in body mass. During the course of the study subjects tended to eat more frequent meals but meal size was reduced due to a more rapid increase in satiety.

A factor likely contributing to suppression of appetite as well as increased basal metabolic rate at altitude is leptin. Leptin is an adipocyte derived hormone which functions as a mediator in the negative feedback control from adipose tissue to the hypothalamus. Leptin circulates at a plasma concentration proportional to body fat mass and controls body weight by reducing food intake and increasing energy expenditure (49).

Circulating leptin is transported across the blood brain barrier where it binds to key hypothalamic neuronal sites such as in the arcuate nucleus and specifically on the pro-opiomelanocortin (POMC) neuron. This interaction leads to release of the POMC-derived neuropeptide, α-melanocyte stimulating hormone (α-MSH) which in turns binds to melanocortin 4 receptors in the paraventricular nucleus. The POMC/ MC4-R system mediates leptin signaling to induce anorectic effects. MC4R activation also increases peripheral sympathetic nerve activity, probably by both direct and indirect signaling processes, leading to increased expression and activity of mitochondrial uncoupling protein 1 (UCP-1) in brown adipose tissue (50). This protein uncouples oxidative phosphorylation causing an increase in thermogenesis and increased energy expenditure.

Hypoxia is a potent stimulus for increased leptin gene expression and subsequent secretion by fat cells. The leptin gene contains several HIF response elements in the promoter region suggesting hypoxia induced stimulation is mediated by a direct effect of HIF in promoting gene transcription. Measurement of leptin levels with ascent to altitude has shown conflicting results (52,53). Some of this discrepancy may be methodological in nature since leptin is secreted in a diurnal fashion and is subject to feedback regulation. As a result values are likely to vary as to when the specimen is taken and whether the subject was at altitude acutely or chronically. In two independent studies ascent to altitude was associated with increased levels

and was more pronounced in those with loss of appetite (52). Reports describing either a decrease or no change in levels are noteworthy in that body weight was reduced. Any loss in body weight as one ascends into altitude is initially due to a preferential loss in body fat. Since leptin is primarily secreted by fat cells, loss of body fat can mask the stimulatory effect of hypoxia on leptin production. In this regard, leptin levels are likely to be greater in subjects suffering weight loss at altitude as compared to subjects who have lost a comparable amount of weight at sea level (54).

Another factor that may limit increases in circulating leptin levels with altitude relates to the stimulatory effect of leptin on sympathetic nerve activity (55,56). Soon after arrival to altitude sympathetic nerve activity is increased and remains so even in well acclimatized subjects (57,58). Using peroneal microneurography sympathetic nerve discharge remains elevated in subjects after four weeks at an altitude of 5260 meters as compared to values obtained at sea level. Administration of oxygen in order to eliminate chemoreflex activation or saline to reduced baroreflex deactivation has only a minor effect in reducing sympathetic activity leaving the underlying mechanism of the chronic over activity unexplained. It is interesting to speculate the tonic increase in sympathetic activity is a marker of increased leptin signaling.

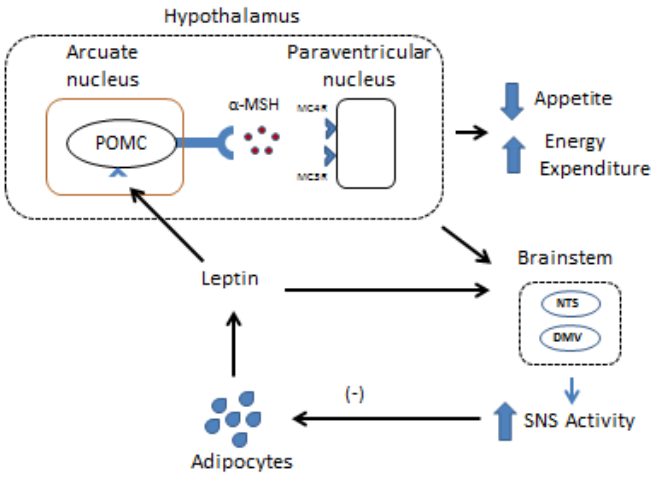

Figure 3. Leptin-melanocortin activation and regulation of appetite and energy expenditure

The degree to which sympathetic nerve activity is increased as a result of leptin signaling is subject to negative feedback since catecholamines inhibit leptin gene expression through β-adrenergic receptors (59). In one study circulating catecholamines and leptin levels were found to be inversely related (60). Some of the reported differences in leptin levels may represent individual variability in the competing effects of hypoxia induced leptin release and feedback control by sympathetic nerves. In this regard the amount of body fat may be a determinant as to the net effect on leptin release. When obese and lean rats are exposed to hypoxia there is a similar increase in hematocrit and gene expression of vascular endothelial growth factor (VEGF) (61). Despite this similar hematologic response obese rats manifest a much greater increase in plasma leptin levels in comparison to lean animals. Since the response of fat cells to catecholamines is decreased in obese animals as a result of altered β-adrenergic signaling, the greater increase in leptin may be the result of less feedback inhibition by sympathetic nerves in the obese as compared to more complete inhibition in the lean animals (62).

In addition to HIF-induced stimulation of leptin, decreased appetite in hypobaric hypoxia may also be the result of a direct effect of HIF in the hypothalamus. The HIF-2α isoform is expressed in the arcuate nucleus of the hypothalamus and has been shown to function as a

nutrient sensor via activation of the POMC neurons (63). Glucose feeding up regulates hypothalamic HIF-2α by inhibiting PHD-mediated hydroxylation of the protein. The stabilization of HIF-2α leads to up regulation of POMC through a direct effect on gene transcription thus providing a mechanism leading to decreased energy intake. When HIF-2α is specifically deleted in the hypothalamus with use of lox/cre methodology, animals develop positive energy balance and obesity. By contrast, over expressing HIF-2α in the arcuate nucleus using a lentiviral co-expression system, animals develop a hypermetabolic phenotype with resistance to the development of obesity upon challenged with a high fat diet.

Adverse Effects of HIF Activation on Exercise Performance

HIF-induced changes in cellular energetics come at the expense of less ATP production and therefore can lead to more rapid fatigue in exercising muscle. The adverse effects of a persistent increase in HIF activity on exercise performance is seen in patients with Chuvash polycythemia (64,65). This autosomal recessive disorder is due to a mutation in the *VHL* gene resulting in an inability of VHL to bind to HIF-α. This results in stabilization of the HIF complex and increased expression of HIF-target genes under normoxic conditions.

Clinically these patients are polycythemic and have increased pulmonary artery pressures and reduced systemic arterial pressure. With exercise these patients demonstrate early and marked depletion of phosphocreatinine and develop acidosis in skeletal muscle as measured with ^{31}P magnetic resonance spectroscopy (64). Lactate accumulation in blood is significantly greater and maximum exercise capacity is significantly reduced when compared to normal controls. In muscle biopsy specimens mRNA expression for PDK is increased along with increased expression of phosphofructokinase and pyruvate kinase. Upregulation of these enzymes are consistent with a shift in energy metabolism away from oxidative phosphorylation suggesting these patients are limited in the capacity to utilize oxygen in response to exercise.

In a separate study random glucose levels and glycosylated hemoglobin A1c levels were reduced in these subjects as compared to normal subjects from the same geographical region (65). Studies in mice with the same VHL mutation also demonstrate a reduction in glucose as well as lower glucose excursions consistent with increased skeletal muscle uptake and glycolysis.

Studies in mice in which the HIF gene is specifically deleted from skeletal muscle further demonstrate the importance of HIF activity on exercise capacity. HIF-1α KO mice were found to have greater exercise endurance as measured by a swimming endurance test or runtime on a treadmill as compared to wild type controls (66). Measurement of glycolytic and mitochondrial enzyme activity as well as decreased amounts of lactate in the serum of exercising HIF-1α KO mice suggest these animals have increased activity of oxidative pathways in muscle.

In these same HIF-1α KO mice baseline oxidative capacity as measured by the respiratory exchange ratio is similar to wild type animals who have been subjected to an endurance training protocol (67). In addition the untrained KO mice have an increased capillary to muscle fiber ratio. Expression of PDK I is decreased in the KO mice suggesting metabolism is being directed towards aerobic metabolism. These data suggest removal of HIF-1α leads to adaptation in skeletal muscle reminiscent of endurance training.

Genetic Variability in HIF activation with Altitude

Even in the absence of a specific mutation or genetic manipulation, variability in HIF-mediated changes in oxygen delivery and utilization are likely to influence the response to and

performance at altitude. Genes specifically involved in the HIF pathway have been identified as contributing to the adaptive changes which characterize high-altitude populations. For example low landers ascending to altitude develop elevations in hemoglobin and hematocrit that are typically higher in comparison to native highlanders (68). Tibetans living at high altitude have hemoglobin levels similar to those expected at sea level and on climbing expeditions develop much less of an increase in comparison to non-native team members.

Studies utilizing a genomic and candidate gene approach comparing Tibetan highlanders and lowland Han Chinese suggest an evolutionary adaption in HIF-mediated affects regarding erythropoiesis (69-71). In these studies a significant divergence was noted in the allelic frequency of single-nucleotide polymorphism (SNP) alleles located in or near the *HIF-2α/EPAS1* and *PHD2/EGLN1* genes. The frequency of these alleles correlated with lower hemoglobin levels noted in the Tibetans. Since activation and stabilization of HIF-2α is the primary mediator of increased erythropoietin production in response to hypoxia these findings suggest the *HIF-2α/EPAS1* and *PHD2/EGLN1* alleles are responsible for the reduced hemoglobin concentration.

This divergence in alleles may have been the result of natural selection whereby the Tibetan *HIF-2α* and *PHD2* genes confer a survival advantage at high altitude by facilitating a blunted hematologic response to hypobaric hypoxia. A blunted rise in hemoglobin is likely to be a favorable adaptation since polycythemia increases blood viscosity and adversely affects microcirculatory blood flow (72). Tibetan highlanders are more resistant to chronic mountain sickness or Monge's disease in comparison to the Han Chinese. This disorder is characterized by excessive erythrocytosis resulting is adverse effects on cardiovascular function due to increased blood viscosity.

An association between hemoglobin concentration and haplotype variation in the nuclear receptor peroxisome proliferator activated receptor-α gene (*PPARA*) has also been identified in Tibetan highlanders (71). Hypoxia has been shown to both decrease and increase PPARα activity depending on the tissue examined. PPARα interacts with the HIF pathway to influence the expression of a number of proteins associated with fatty acid oxidation. In a manner analogous to the effect on hemoglobin, the shift toward anaerobic metabolism under the dictates of HIF may be attenuated such that aerobic metabolism via oxidative phosphorylation is better preserved in Tibetans. This difference would allow a greater amount of ATP to be generated for exercising muscle and in part account for the superior performance climbing Sherpas exhibit on high altitude expeditions. In this regard enzyme activity measured in Sherpa muscle biopsy specimens show low activity of LDH relative to pyruvate kinase suggesting muscle metabolism is poised to burn carbohydrate to CO_2 and H_2O as opposed to lactate (73). In addition, the intensity of exercise at which point lactate begins to accumulate in the blood (lactate threshold) is greater in Sherpas when compared to lowlander populations.

Minimal change in body weight in Sherpas despite prolonged stays at high altitude may also be a reflection of an attenuated HIF effect on metabolism. As mentioned previously non-adapted lowlanders ascending to altitude typically lose weight initially accounted for by loss of fat mass and followed by muscle wasting at more extreme elevations (37). Sherpas do not exhibit this pattern. In the American Medical Research Expedition to Everest (AMREE) study Sherpas who arrived at base camp with half as much body fat as compared to their Western counterparts were able to maintain body weight during prolonged residence above 5400 meters. Limb circumference remained the same in the Sherpas as compared to a fall in the Westerners indicating a preservation of muscle mass in the Sherpas.

Sexual Dimorphism in HIF Activation at Altitude

Differences in metabolism between men and women at altitude suggest there may be a sexual dimorphism regarding the effects of HIF. HIF normally results in a shift of metabolism favoring the utilization of glucose as a substrate. In two separate studies of men taken to 4300 meters, whole body glucose uptake and glucose extraction was significantly increased as compared to sea level values (15,16). This increase was detected within hours of arrival and remained elevated after 21 days of exposure. By contrast, substrate preference during exercise differs in women both at sea level and at altitude. Under normoxic conditions studies examining substrate utilization during endurance exercise show women derive a greater proportion of energy from utilization of fat and catabolize less carbohydrate as compared to equally trained and nourished men. This same constraint on carbohydrate metabolism has been demonstrated in women taken to 4300 meters (74). After 10 days of exposure blood glucose utilization rates were lower as compared to values measured at sea level. Whole body carbohydrate utilization as determined by measurement of the respiratory exchange ratio was also lower in comparison to sea level values when measured at rest or during exercise. Studies in female rats under sea level conditions and after exposure to simulated altitude also failed to show a shift towards greater utilization of carbohydrates under conditions of hypobaric hypoxia (75).

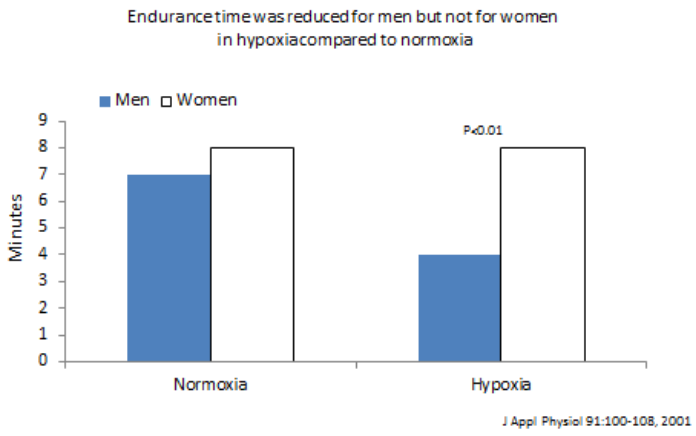

Figure 4. Adductor pollicis muscle endurance time to exhaustion in men and woman measure in normoxic and hypoxic conditions

A preferential shift in metabolism away from glucose and towards fatty acid oxidation has the potential to provide greater amounts of ATP and could translate into better exercise performance in women as compared to men under hypoxic conditions. In this regard, performance was compared between men and women undergoing high-intensity intermittent static contractions of the adductor pollicis muscle at sea level and shortly after arrival to 4300 meters (76). Under conditions of hypobaric hypoxia performance was less impaired in women. In particular, women did not display the increase in overall fatigue rate and decrease in endurance time to exhaustion found in men.

Changes in body weight at altitude also tend to be less in women as compared to men. In eight college women residing at 4300 meters for 2.5 months a decrease in skin fold thickness and reduction in limb circumference was noted but little change in body weight (77). The authors concluded that women required no more energy intake at high altitude to maintain body weight as compared to sea level. When 12 women were taken to 5050 meters for 21 days, mean body mass did not change and there was no significant difference in fat or fat free mass compared to baseline values (78). Measurement of basal metabolic rate is increased by 6.9% above sea level by day 3 at an altitude of 4300 meters but falls to sea level values by day six (79). This transient and short lived increase is in contrast to the greater and more prolonged increase in basal metabolic rate described in men at a similar altitude.

Attenuation in HIF activation in response to hypobaric hypoxia in woman is an attractive way to account for the gender differences in glucose utilization, exercise performance, body weight change, and change in basal metabolic rate noted above. In a number of settings estrogen has been shown to downregulate HIF. In ovariectomized female rats there is increased protein expression of HIF-1α in periaortic fat as compared to controls (80). These animals develop features of metabolic syndrome to include increased body weight and lipids and insulin resistance. Administration of 17-β-estradiol to the ovariectomized animals leads to a reduction in body weight accompanied and an improvement in the features of the metabolic syndrome. Western blot and immunohistochemical staining shows HIF-1α is reduced to control values in the periaortic fat of estrogen treated animals. In a rat model of obstructive sleep apnea in which animals are exposed to intermittent bouts of hypoxemia, administration of estrogen was found to significantly attenuate the fatigue measured in the genioglossus muscle (81). Both mRNA and protein levels of HIF-1α were increased in intermittent hypoxia animals as compared to controls. Administration of estrogen was found to decrease both gene expression and protein levels of HIF-1α in a dose dependent manner.

Physiologic does of 17-β-estradiol has been shown to attenuate hypoxia-induced erythropoietin gene expression by interfering with hypoxic increase in HIF-1α levels and activity (82,83). This inhibitory effect on HIF-1α can be blocked in the presence of an estrogen receptor antagonist. The ability of estrogen to downregulate HIF activity in the setting of hypoxia may be of relevance to the excessive erythrocytosis which characterizes chronic mountain sickness. Chronic mountain sickness is rare in premenopausal women and increases in frequency after menopause (84,85). In animal models of hypoxemia, females develop less severe pulmonary hypertension, right ventricular hypertrophy, and polycythemia compared to males (82).

To summarize, hypobaric hypoxia provides key metabolic regulatory components which facilitate weight reduction. Specifically, hypoxia not only suppresses appetite but it also uniquely increases energy expenditure. These metabolic changes can be explained by up-regulation of a transcription factor, hypoxia inducible factor, or HIF. The degree to which altitude leads to weight reduction is dose dependent; with modest altitude, loss of weight is primarily comprised of fat with sparing of lean muscle mass. At more extreme altitudes muscle wasting begins to occur. This dose dependency in alterations in body composition may be related to a greater degree of HIF signaling at extreme altitude. Changes in metabolism brought about by HIF while beneficial for weight reduction, may come at the expense of reductions in exercise performance. Genetic factors and sexual dimorphism may account for the variability in the degree to which HIF is activated in response to altitude and may explain differences in weight reduction and exercise performance noted in different populations. While exposure to altitude may not be a feasible strategy for weight reduction in the population at large, greater understanding of the mechanisms brought about by HIF activation may allow for development of more efficacious pharmacologic interventions.

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