HONORS THESIS

HYPOXIA-INDUCIBLE FACTOR-1α SIGNALING IN CARDIAC RESPONSES TO HYPOBARIC HYPOXIA

Submitted by

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ABSTRACT

HYPOXIA-INDUCIBLE FACTOR-1α SIGNALING IN CARDIAC RESPONSES TO HYPOBARIC HYPOXIA

Previous data from the Chicco lab demonstrated that skeletal muscle metabolic adaptations to hypobaric hypoxia (HH) involves glucocorticoid receptor (GR) signaling, but not hypoxia inducible factor (HIF)-1α. The present study investigated whether the same is true in cardiac muscle. Male F344 rats were given a glucocorticoid receptor blocker (RU486) or no drug beginning five days before and throughout a fifteen-day exposure to 17,200 feet simulated in a hypobaric chamber or remained at Fort Collins elevation at 5,003 feet. Cardiac tissue was collected and analyzed for expression of GR, HIF1-α, VLCAD (a fatty acid oxidation enzyme), and each of the five complexes involved in (OXPHOS) oxidative phosphorylation. Current data indicates that while HH had no effect on cardiac GR expression, HIF1-α protein levels were elevated in the right ventricle (RV) and left ventricle (LV). Consistent with this finding, VLCAD expression was lower in the RV, with no effect of RU486 treatment, while the opposite trend was previously seen in skeletal muscle. Taken together, our results thus far indicate that in contrast to skeletal muscle, HIF1-α, rather than GR signaling, may play an important role in cardiac responses to HH.

ACKNOWLEDGMENTS

I would like to thank my honors thesis advisor Dr. Adam Chicco for his guidance and support through my undergraduate years. I would also like to thank Dr. Ryan Maresh for his support and expertise on my thesis committee as well as Luke Witcomb, M.S. for his mentorship and instruction with western blotting.

INTRODUCTION

Hypoxemia, or low blood oxygen levels, is induced by exposure to hypobaric hypoxia at high altitude or impairment of cardiopulmonary gas exchange, which decreases oxygen supply to body tissues. (Sarkar, Niranjan, Banyal, 2017). How tissues respond to chronic hypoxemia to maintain their function is incompletely understood, especially regarding cardiac ventricular tissue. Previous work in our lab indicates that skeletal muscle (white gastrocnemius and plantaris) metabolic responses to chronic hypobaric hypoxia (HH) involves glucocorticoid receptor (GR) signaling. The current study determined whether the same occurs in the heart, where oxygen supply and utilization are crucial for survival.

HYPOXIA BACKGROUND

Research surrounding hypoxia, a physiological phenomenon characterized by lower levels of tissue oxygenation, has drastically increased since the 2019 Nobel Prize in Physiology was awarded to William G. Kaelin Jr., Sir Peter J. Ratcliffe, and Gregg L. Semenza for their work on cellular adaptation to oxygen availability by discovery of the Hypoxia-Inducible Factor (Sarkar et al., 2017; Richalet et al., 2024; Lee et al., 2020). There are multiple types of hypoxia; hypobaric hypoxia is a reduction in barometric pressure resulting in a lower partial pressure of oxygen in the air; hypemic hypoxia is the reduced ability of hemoglobin within red blood cells to bind O2; and histotoxic hypoxia is the inability of cells within tissues to utilize O2 (Bhutta, Alghoula, Berim, 2024). It is important to note that the percentage of oxygen, or fraction of inspired oxygen (FiO2), in the air is constant around 21% regardless of altitude (Fuentes, Chowdhury, 2022). Oxygen is crucial for every human cell; any reduction in oxygen availability has multi-system effects on physiological functions (Richalet, Hermand, Lhuissier, 2024). Nucleated cells can sense oxygen concentration and initiate both acute and long-term response mechanisms, which include gene transcription through a major transcription factor−HIF-1α−which will be discussed in detail (Michiels, 2004). Oxygen homeostasis is vital for cellular function as diatomic oxygen serves as the final electron acceptor of the electron transport chain playing a crucial role in ATP production (Semenza G.L., 2012). Systemic effects of hypoxia include cardiovascular, pulmonary, metabolic, hematological, and endocrine effects that illicit specific responses that will be explored in depth (Richalet et al., 2024).

ACUTE HYPOXIA EXPOSURE

Acute exposure to high-altitude hypoxic environments results in several physiological changes to respond to the lower partial pressure of oxygen (PO2), including activation of the sympathetic nervous system, tachycardia (rapid heart rate), increased ventilation rate, increased cardiac output and cardiac contractility, a slight increase in blood pressure, a decrease in stroke volume, and higher pulmonary artery pressure due to hypoxic pulmonary vasoconstriction (Richalet et al., 2014; Naeije, 2010; Bärtsch, Gibbs, 2007). As a result of the stress placed on the body during hypoxic conditions, the hypothalamo-pituitary-adrenocortical (HPA) axis is activated (Herman et al., 2016). The HPA axis first triggers the hypothalamus to release corticotropin releasing hormone (CRH) which subsequently triggers adrenocorticotrophic hormone (ACTH) release into systemic circulation by the anterior pituitary (Herman et al., 2016). ACTH causes synthesis and secretion of glucocorticoids (including cortisol, the primary human glucocorticoid) from the adrenal cortex (Herman et al., 2014). We confirmed the activation of the HPA axis in our study by measuring the presence of plasma corticosterone (the primary glucocorticoid in rodents) within the bloodstream of the rats as seen in Figure 1A. (Richalet et al., 2014).

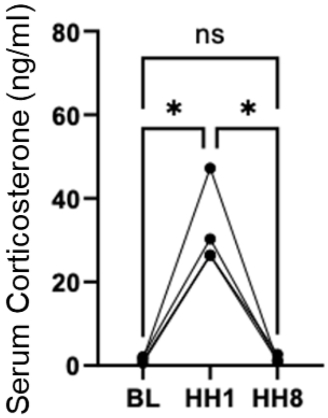

Figure 1 A). Elevated serum corticosterone levels in the tail vain of rats exposed to HH. *Luke Whitcomb, M.S., 2022.*

Within the coronary circulation, acute exposure to hypoxia results in coronary arterial dilation in order to compensate for hypoxemia (Bärtsch et al., 2007). On a systemic level, vasodilation of arterioles occurs within the first few hours of exposure to hypoxia, then due to sympathetic activation releasing catecholamines (epinephrine and norepinephrine) into the bloodstream, systemic vasoconstriction will occur from then on (Bärtsch et al., 2007). The rise in sympathetic activation throughout hypoxia exposure accounts for the increase in blood pressure and systemic vascular resistance seen in chronic exposure (Bärtsch et al., 2007). The lower stroke volume observed during acute altitude is thought to be due to the reduction in LV filling pressure (Bärtsch et al., 2007). Reduced LV filling pressure may be caused in part by the increase in RV pressure and subsequent dilation due to increased pulmonary vascular resistance (Bärtsch et al., 2007).

Hypoxemia, the reduction blood oxygen levels, is commonly caused by ventilation/perfusion (V/Q) mismatch (Sarkar et al., 2017). A lower V/Q ratio, or decreased ventilation and/or increased perfusion, induces hypoxemia and is present in conditions such as pulmonary edema (Bhutta et al., 2024). A higher V/Q ratio, characterized by decreased perfusion and increased ventilation, can also induce hypoxemia in conditions such as emphysema (Bhutta et al., 2024). During hypoxic pulmonary vasoconstriction (HPV), areas of low ventilation in the lungs constrict, diverting blood flow to areas of higher ventilation in an attempt to equalize ventilation and perfusion rates (Sarkar et al., 2017). Vascular smooth muscle cells are able to respond acutely at a local level to hypoxic conditions via HPV (Michiels, 2004). Hypoxic pulmonary vasoconstriction occurs through a debated mechanism of inhibition of O2 sensitive potassium (K+) channels on the cell membrane, altering membrane potential to a more depolarized state where calcium (Ca2+) can flood into the cell and initiate constriction (Michiels, 2004).

Hypoxemia is immediately sensed by the carotid bodies within the bifurcation of the common carotid artery and neuroepithelial bodies within intrapulmonary airways (Michiels, 2004). The carotid bodies sense hypoxemia within the arterial circulation, and through synaptic communication with the carotid sinus nerve are able to enact an increase in heart rate (Michiels, 2004). The neuroepithelial bodies synapse with fibers of Vagus CNX in order to increase ventilation rate (Michiels, 2004). Hypoxemia leads to closure of O2 sensitive potassium (K+) channels on the surface of these chemoreceptor cells, resulting in depolarization and opening of voltage-gated calcium (Ca2+) channels allowing Ca2+ to enter the cell and activate neurotransmitter release to communicate with sensory fibers of the two nerves involved (Michiels, 2004). Once stimulated, the carotid sinus nerve releases norepinephrine acting as a neurotransmitter which will bind to β-1 adrenergic receptors to stimulate cardiac inotropy (Michiels, 2004). Another endogenous stress response system that is activated alongside the SANS is the HPA axis as mentioned above (Herman et al., 2014). These two systems allow for widespread systemic adaptation to hypoxemia and research indicates interconnections between the two (Rotenberg, McGrath, 2016). Hypoxemia results in release of CRH specifically from *tractus solitarii* neurons in the paraventricular nucleus of the hypothalamus (Herman et al., 2014). These neuronal afferents project into the locus coeruleus, an area in the pons that serves as the main source of norepinephrine in the brain, while at the same time noradrenergic neurons from the locus coeruleus project into the paraventricular nucleus (Rotenberg, McGrath, 2016). CRH causes firing of the locus coeruleus neurons and subsequent noradrenaline (norepinephrine) release (Rotenberg, McGrath, 2016). In a reciprocal fashion, noradrenaline increases CRH mRNA expression within the paraventricular nucleus (Rotenberg, McGrath, 2016). Cortisol is also observed to amplify sympathetic responses to stress in the cardiovascular system, including heart rate (Rotenberg, McGrath, 2016).

LONG-TERM HYPOXIA EXPOSURE

Long-term exposure to hypobaric hypoxia triggers additional responses, including downregulating adrenergic receptors in a cardioprotective manner, increased bicarbonate excretion in urine, erythropoiesis stimulation, and inhibition of the renin-angiotensin-aldosterone system (RAAS) (Richalet et al., 2024). Blood pressure and systemic vascular resistance remain elevated throughout hypoxia exposure whereas maximal cardiac output decreases upon acclimatization (Bärtsch et al., 2007; Naeije, 2010). Resting heart rate remains elevated resulting in continued reduction of stroke volume throughout chronic HH exposure (Naeije, 2010). Myocardial oxygen extraction, the ratio of global O2 consumption to global O2 delivery, must increase in order to maintain adequate oxygenation during chronic HH (Bärtsch et al., 2007).

Higher hematocrit, the ratio of red blood cell volume to total blood volume, is classically observed following prolonged exposure to hypoxic environments (Villafuerte et al., 2022), as demonstrated in Figure 1B from our study in rats exposed to 15 days of simulated exposure to 17,200 feet in a hypobaric chamber below. This is classically due to hypoxemia stimulating HIF-modulated activation of the erythropoietin gene responsible for increasing red blood cell production (Zhao et al., 2023), but is also partially dependent upon GR signaling indicated in Figure 1B by a significant decrease in the response induced by treatment with the GR blocker RU486 in rats.

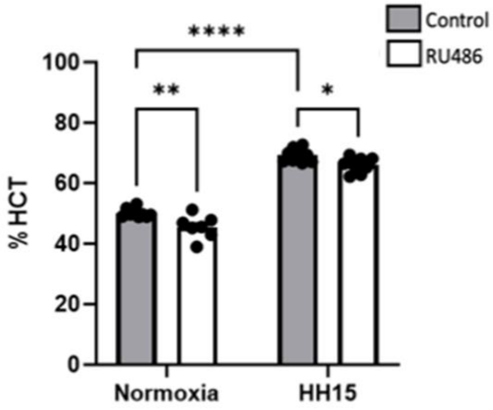

Figure 1. B) Elevated hematocrit levels observed in rats exposed to HH with a decrease in response in rats treated with RU. *Luke Whitcomb, M.S., 2022.*

β-adrenergic receptors, the primary receptors binding catecholamines, as well as adenosine A1 receptors are downregulated in cardiomyocytes in response to long-term elevated levels of norepinephrine and adenosine to lower heart rate and cardiac inotropy (Richalet et al., 2024). This desensitization of G-protein coupled receptors during chronic hypoxia occurs by lower expression of the Gs protein and higher expression of the Gi protein that are responsible for coupling -adrenergic receptors to adenylate cyclase, resulting in lower cyclic AMP (cAMP) production (Richalet et al., 2024). This occurs in order to attenuate the effects of the sympathetic nervous system (Richalet et al., 2024).

The increased ventilation rate observed upon exposure to HH leads to a condition called respiratory alkalosis, marked by insufficient CO2 levels in the blood (Brinkman, 2023). Looking at the bicarbonate buffer system equation,

$$CO2 + H2O \leftrightarrow H2CO3 \leftrightarrow H^{+} + HCO3^{-}$$

Decreased CO2 content will result in higher circulating bicarbonate ($HCO3^{-}$) levels and will increase blood pH (Brinkman, 2023). As mentioned above, long-term hypoxia exposure will increase renal bicarbonate excretion in urine in order to maintain a physiological pH of 7.35-7.45 (Brinkman, 2023).

HYPOXIA INDUCIBLE FACTOR

The primary regulator of cellular oxygen homeostasis is the heterodimeric transcription factor hypoxia-inducible factor (HIF) (Semenza G.L., 2012). HIF-1 is expressed by all mammalian cells and made of both an unstable α subunit and a stable β subunit (Semenza G.L., 2012). Both subunits are a part of the PAS family, a collection of related "helix-loop-helix" structure transcription factors (Schofield, Ratcliffe, 2004). The HIFβ subunit, the same as the aryl hydrocarbon receptor nuclear translocator (ARNT) gene, is constituently expressed as a nuclear protein (Kaelin, Ratcliff, 2008). The HIFα subunit, on the other hand, has a half-life of less than five minutes and has three known closely related forms; HIF-1α, HIF-2α, and HIF-3α that are hypoxia-sensitive (Schofield, Ratcliffe, 2004; Zhao et al, 2023). Little is known about HIF-2α and HIF-3α (Schofield, Ratcliffe, 2004). HIF-1α contains four domains crucial to its function as the oxygen-sensitive subunit; the amino-terminal activation domain (NAD), the amino terminal oxygen-dependent degradation domain (NODDD), the carboxy-terminal activation domain (CAD), and the carboxy-terminal oxygen-dependent degradation domain (CODDD) (Schofield, Ratcliffe, 2004). Each of these domains play a role in regulating HIF-1 via protein hydroxylation, with NODDD, CODDD, and NAD directly regulating proteolysis (Schofield, Ratcliffe, 2004).

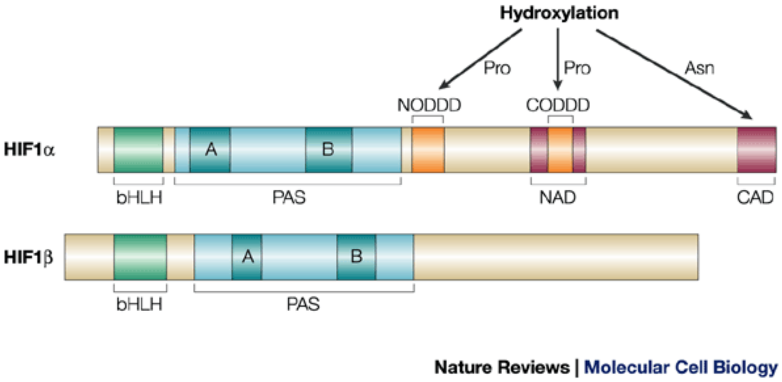

Figure 2: The structural differences between the HIF-1a and HIF-1B subunits and their respective domains. *Nature Reviews, 2004.*

Under normoxic conditions, hydroxylation of specific residues within each domain occurs (Schofield, Ratcliffe, 2004). Hydroxylation is accomplished by HIF hydroxylases (Schofield, Ratcliffe, 2004). Within the NODDD and CODDD domains, hydroxylation of prolyl residues by prolyl hydroxylase domain (PHD) enzymes are responsible for increasing interaction with the von Hippel-Lindau tumor suppressor (pVHL) which aids in tagging HIF-1α for proteolysis (Schofield, Ratcliffe, 2004). Factor inhibiting HIF (FIH), is another HIF hydroxylase that functions to hydroxylate asparagine residues within the CAD domain to block transcriptional activity of HIF-1α (Schofield, Ratcliffe, 2004). The ubiquitin-proteasome pathway is responsible for degradation of the HIF-1α subunit (Schofield, Ratcliffe, 2004). Under hypoxic conditions, prolyl hydroxylation and thereby pVHL interaction is decreased resulting in the HIF-1α subunit being stabilized and building up within the nucleus (Schofield, Ratcliffe, 2004). This hydroxylase inhibition is due to oxygen being a necessary cofactor for PHD activity (Schofield, Ratcliffe, 2004). FIH activity is also inhibited, allowing p300, a co-activator, to associate with HIF-1α resulting in HIF-1α stability and transcriptional activity (Schofield, Ratcliffe, 2004). As HIF-1α accumulates in the nucleus, it dimerizes with HIF1-β to form a DNA-binding HIF complex (Dengler, Galbraith, Espinosa, 2014).

The HIF complex then binds to hypoxia response elements (HREs) that are associated with a large variety of transcriptional target genes that respond to hypoxia (Schofield, Ratcliffe, 2004). HIFs activate production of messengers involved in the body's hypoxia response, including erythropoietin (EPO), endothelin-1 (ET-1), glucose transporters, nuclear factor k-B (NF-kB), nitric oxide synthases (NOS), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) (Richalet et al., 2024). The erythropoietin (EPO) gene contains a hypoxia response element at its 3' end; binding of the HIF complex induces activity of EPO released by the liver to increase production of red blood cells from hematopoietic stem cells within the bone marrow to increase oxygen delivery in a hypoxic environment (Zhao et al., 2023). VEGF is known to be induced by HIF-1α and is responsible for angiogenesis, or the production of blood vessels (Zhao et al., 2023).

HIF AND METABOLISM

HIF-1 also plays a role in regulating cellular metabolism as hypoxia severely negatively impacts mitochondrial respiration (Semenza G.L., 2012). As the heart relies heavily on oxidative metabolism to generate roughly 6kg of ATP required each day for contractility, it is important to understand this process (Knutson et al., 2023). Carbohydrates via glucose, fatty acids, and proteins via amino acids are catabolized to enter the tricarboxylic acid cycle to generate high-energy electron carriers to undergo oxidative phosphorylation (Nolfi-Donegan, D. et al., 2020). Oxidative phosphorylation creates 95% of ATP within cardiomyocytes with fatty acids as the primary substrate (Knutson et al., 2023). Oxidative phosphorylation is comprised of two parts; the electron transport chain and chemiosmosis (Ahmed et al., 2023). The electron transport chain within the inner mitochondrial membrane is responsible for oxidizing the two electron carriers—NADH and FADH2—to transfer their electrons to O2 (Michiels, 2004). Electrons enter at either Complex I or Complex II and are passed through membrane-bound protein complexes in a series of redox reactions (Michiels, 2004). These complexes include: Complex I (NADH:ubiquinone oxidoreductase) which pumps 4 protons into the intermembrane space; Complex II (succinate dehydrogenase) which pumps zero protons into the intermembrane space; Complex III (cytochrome C reductase) which pumps 4 protons into the intermembrane space; Complex IV (cytochrome C oxidase) which transfers the electrons to oxygen and pumps 2 protons into the intermembrane space; and the ATP synthase (Complex V), which harnesses the proton gradient resulting from activities of Complexes I, III, and IV to phosphorylate adenosine diphosphate (ADP) to adenosine triphosphate (ATP) (Nolfi-Donegan, D. et al., 2020; Ahmed et al., 2023).

When tissue oxygen levels are low, HIF-1 upregulates the transcription of genes that encode for glucose transporters, including GLUT-1, as well as glycolytic enzymes such as pyruvate dehydrogenase kinase 1 (PDHK1) (Semenza G.L., 2012). PDHK1 activation thereby inactivates pyruvate dehydrogenase (PDH) and inhibiting its function of converting pyruvate, the end-product of glycolysis, to acetyl-CoA (Semenza G.L., 2012). The depletion of acetyl-CoA subsequently slows the tricarboxylic acid (TCA) cycle and aids in in metabolism switching from an oxidative to glycolytic state (Semenza G.L., 2012). HIF-1 also upregulates the enzyme that converts pyruvate to lactate, lactate dehydrogenase A (LDHA) in hypoxic circumstances (Semenza G.L., 2012). By shifting substrates away from mitochondria and oxidative phosphorylation, there is insufficient electron transport occurring and a reduction in ATP production (Semenza G.L., 2012). The change in metabolism to glycolysis is thought to be the cell's way of preventing an overabundance of reactive oxygen species (ROS) within the mitochondria which would lead to toxicity and cellular damage (Semenza G.L, 2012).

CARDIAC-SPECIFIC HIF EFFECTS

HIFs are central in cardiac responses to ischemia; HIF-1 is observed to also mediate cardioprotective responses as the vast majority of cardiovascular diseases are characterized by a lack of blood flow, oxygen delivery, and tissue ischemia (Knutson et al., 2021). Within coronary artery disease (CAD), atherosclerotic plaques within the coronary arteries of the heart results in a lack of myocardial perfusion, increasing HIF-1 activity (Semenza G.L, 2012). The remodeling of collateral circulation via HIF-1α induced vascular endothelial growth factor (VEGF) expression, is a subsequent response to try and repurfuse the tissue (Semenza G.L, 2012). Before collateral vessels are formed, HIF-1's response of shifting cardiomyocyte metabolism to a glycolytic state provokes a "preconditioning phenomenon" where the heart is exposed to short ischemia/reperfusion cycles (Semenza G.L, 2012). HIF-1α also activates genes encoding enzymes that produce the amino acid adenosine, which serves to facilitate cardiac preconditioning (Semenza G.L, 2012). Under ischemic conditions, HIF-1α specifically is seen to be significantly elevated in the peri-infarct region of cardiac tissue and can increase the amount of inducible nitric oxide synthase (iNOS) to vasodilate and lessen the impacts of ischemia-repurfusion injury (Zhao et al., 2023).

CAD and other cardiovascular diseases can lead to myocardial infarction (MI) characterized by local hypoxia and lack of perfusion (Zhao, Yongchao, 2023). Within a few seconds of coronary occlusion, HIF modulates an increase in anaerobic glycolysis, lowering intracellular pH (Martinez et al., 2017). Extracellular K+ levels rise and cause a lowering of the resting membrane potential, increasing the rate of action potential generation and electrical conduction, which can lead to ischemia-induced arrhythmogenesis (Martinez et al., 2017). Sympathetic activation from ischemic conditions results in lipolysis, or the breakdown of triacylglycerols, to elevate circulating levels of free fatty acids (FFA's) which can lead to cardiac lipotoxicity (Martinez et al., 2017). HIF-1 induced VEGF expression is crucial post-MI to revascularize the area and create collateral vessels via angiogenesis (Zhao et al, 2023).

GLUCOCORTICOID RECEPTOR

Our previous study in skeletal muscle demonstrated the dependence of skeletal muscle adaptation to hypobaric hypoxia on glucocorticoid receptor (GR) signaling. When GR was blocked with RU486, the muscle was unable to adapt successfully to full functionality and exercise at hypoxia. GR, a ligand-activated transcription factor, and HIF signaling are thought to be closely tied as glucocorticoids are involved in the development of cardiovascular diseases (Liu et al., 2019; Marchi et al., 2021). Glucocorticoids such as cortisol are steroid hormones synthesized by the adrenal cortex and released both under stressful conditions and in a circadian manner (Liu et al., 2019). The tissue-specific metabolic enzyme 11B-HSD2 has limited activity in the vasculature, meaning conversion of active cortisol to inactive cortisone is reduced (Liu et al., 2019). Because of this, circulating cortisol has a direct effect on the cardiovascular system (Liu et al., 2019). GR signaling is also shown to play a role in cardiac contractility through potential regulation of L-type Ca2+ channels (Liu et al., 2019). Studies have demonstrated that

GR is present in both vascular endothelial cells and vascular smooth muscle and therefore are involved in blood pressure regulation (Liu et al., 2019). Because of glucocorticoid’s (GC) anti-inflammatory affects, synthetic GC’s are often used to treat conditions linked to hypoxia (Marchi et al., 2021). The interactions between HIF and GR signaling in the cardiovascular system are not well studied; some demonstrate GR signaling promoting or inhibiting HIF activation while others show HIF to dampen the GC response to inflammation (Marchi et al., 2021). Overall, both HIF and GR signaling are known to regulate tissue homeostasis and responses to cellular stress through various mechanisms including metabolic regulation (Marchi et al., 2021).

MATERIALS AND METHODS

To investigate the role of HIF and GR signaling in the right and left ventricles, male F344 rats were exposed to fifteen days of HH simulating 17,200 feet above sea level in the CSU Hypobaric Chamber Facility or to normoxia (Fort Collins elevation; 5,003 feet) with or without pharmacologic GR blockade with RU486 (60mg/kd/d in chow beginning five days prior to HH exposure). Skeletal muscle (white gastrocnemius and red soleus) and cardiac tissues (right and left ventricles) were weighed and analyzed for protein expression of GR, HIFI- α, and VLCAD by western immunoblotting.

RESULTS

Chronic HH induced RV hypertrophy regardless of whether GR signaling was inhibited by RU486 but had no effect on LV mass. Cardiac GR protein levels were not statistically affected by HH or GR blockade treatment in the LV or RV:

☐ Control
▨ RU486

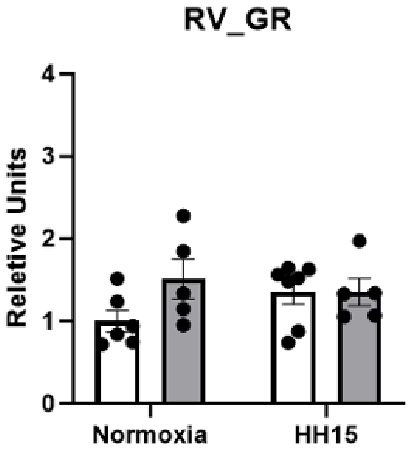

Figure 3. A) No consistent effect in RV tissue of HH or RU486 treatment on GR expression. *Luke Whitcomb, M.S., 2024.*

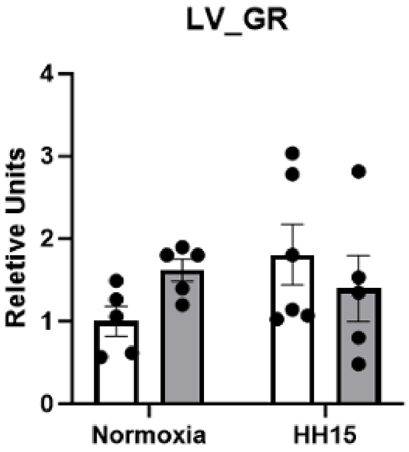

Figure 3. B) No consistent effect in LV tissue of HH or RU486 treatment on GR expression. *Luke Whitcomb, M.S., 2024.*

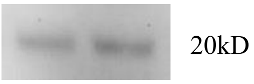

Figure 3. C) Chemiluminescent image of control vs. HRU bands show no difference between control band (left) and treatment band (right) for GR in the RV.

GR is elevated in the white gastrocnemius skeletal muscle, but not the plantaris muscle; this may be due to the differences in nature between the two fiber types:

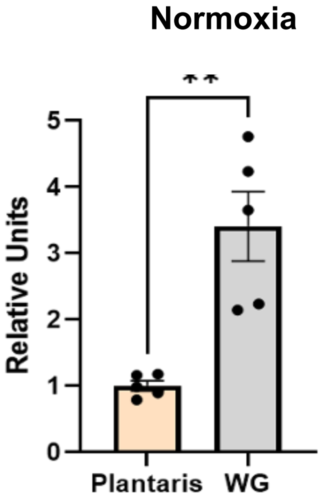

Figure 4. A) GR levels are significantly elevated in the white gastrocnemius muscle in normoxia. *Luke Whitcomb, M.S., 2022.*

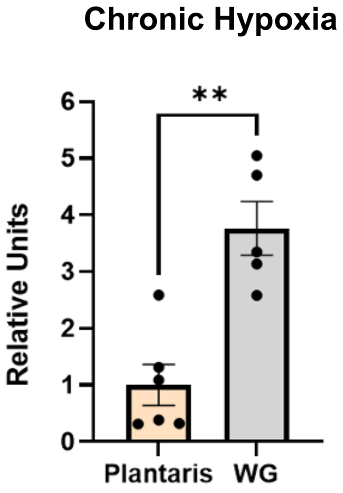

Figure 4. B) GR levels are significantly elevated in the white gastrocnemius muscle in chronic hypoxia. *Luke Whitcomb, M.S., 2022.*

HIF-1a protein tended to be elevated by chronic HH in the RV and LV, but was not significantly elevated in chronic HH in skeletal muscle:

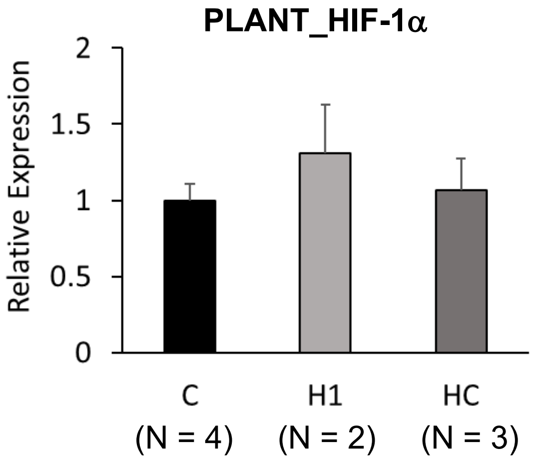

Figure 5. A) Acute HH tends to increase HIF-1α in Plantaris muscle, but the signal is underpowered.

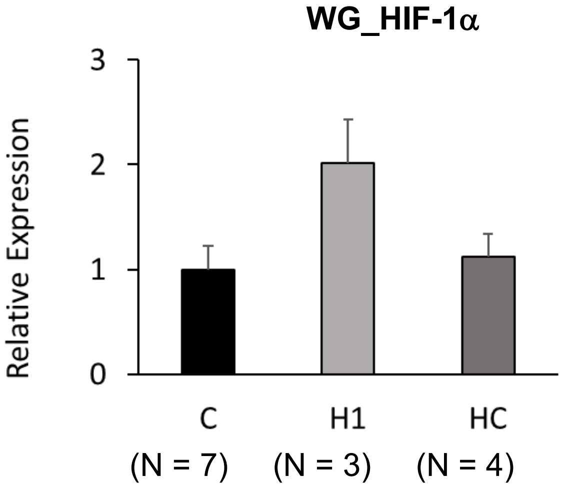

Figure 5. B) Acute HH tends to increase HIF-1α in WG muscle, but the signal is also underpowered.

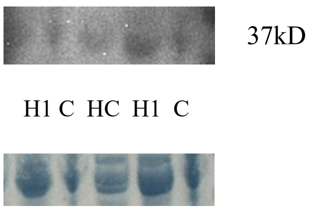

Figure 5. C) Chemiluminescent (top) and amido black images (bottom) of weak Plantaris HIF-1α bands.

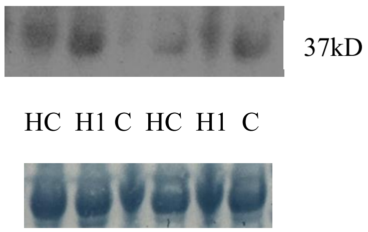

Figure 5. D) Chemiluminescent (top) and amido black images (bottom) of weak WG HIF-1α bands.

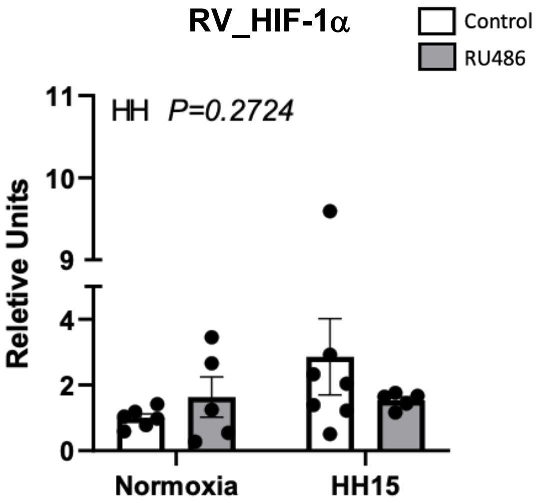

Figure 4. A) HIF-1α concentrations in the RV tissue trend higher in hypoxia.

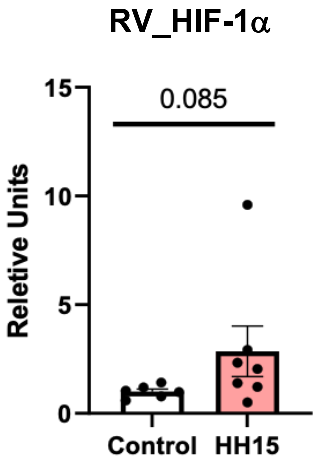

Figure 4. B) 1-tailed t-test of RV HIF1-α concentrations.

Figure 4. C) Chemiluminescent image of control vs. HRU bands show significantly higher HIF-1α expression in the RV upon analysis.

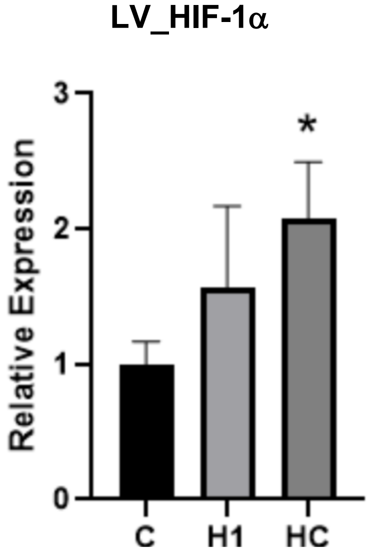

Figure 5. A) HIF-1α expression is significantly elevated in the LV of rats exposed to chronic HH.

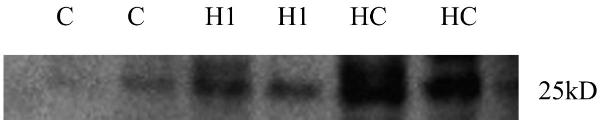

Figure 5. B) Chemiluminescent image of LV HIF-1α bands.

Figure 5. C) Amido black image of LV HIF-1α bands in the same order demonstrating equal protein loading.

Chronic HH decreased expression of cardiac very-long chain acyl-CoA dehydrogenase (VLCAD), a fatty acid oxidation enzyme, in the RV. VLCAD expression may be attenuated by GR blockade in the LV. The opposite trend was seen in muscle, where VLCAD expression was higher in white gastrocnemius muscle:

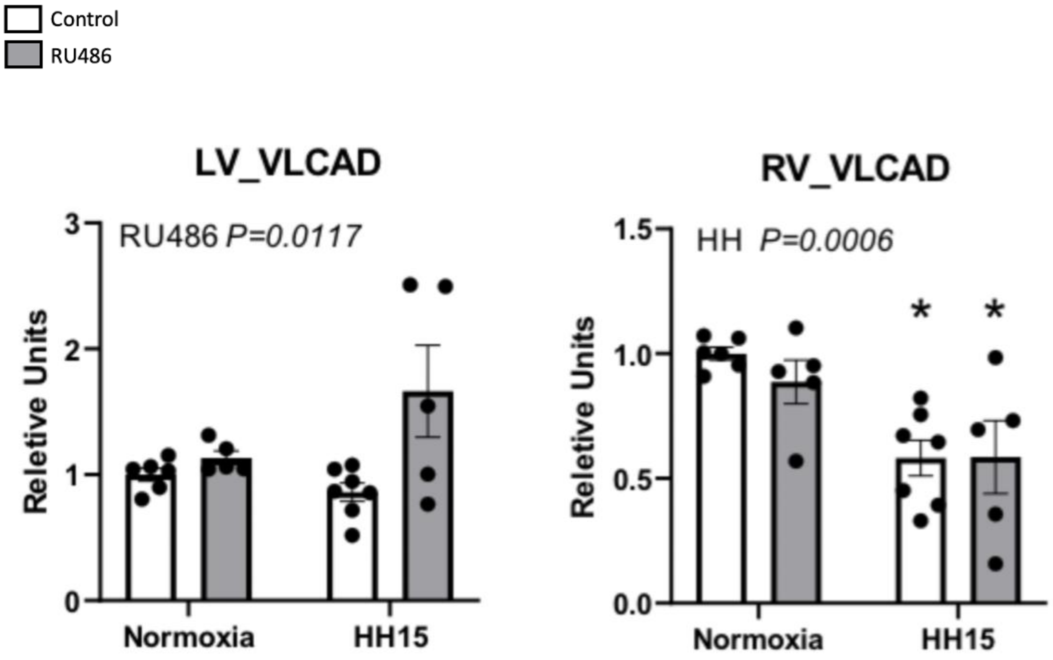

Figure 6. A) VLCAD concentrations in the LV exhibit no significant difference between normoxia and hypoxia groups.

Figure 6. B) VLCAD enzyme levels in RV tissue in the hypoxia groups are lower than normoxia with no consistent effect of GR blockade.

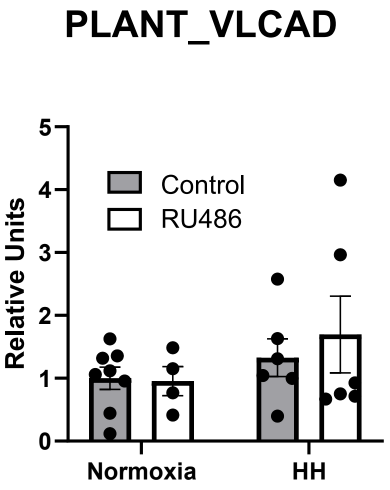

Figure 7. A) VLCAD levels are not significantly elevated within the plantaris muscle under HH, irrespective of RU treatment. *Luke Whitcomb, M.S., 2022.*

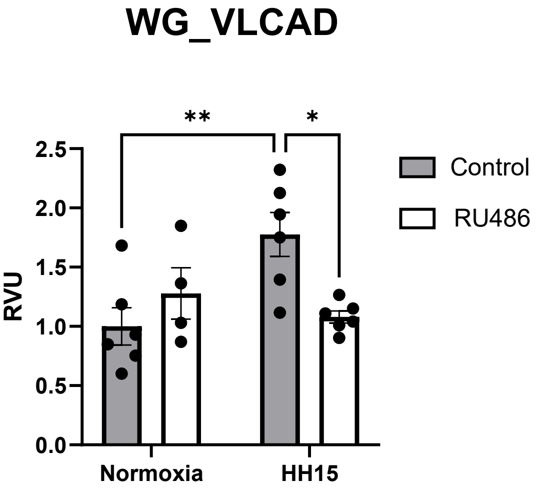

Figure 7. B) VLCAD levels are significantly elevated in the WG muscle in HH and attenuated by GR blockade in HH. *Luke Whitcomb, M.S., 2022.*

DISCUSSION

Our present results suggest that there are fundamental differences between the mechanisms by which skeletal and cardiac muscle metabolism respond to hypobaric hypoxia. Cardiac responses to chronic HH appear to be independent of GR signaling but vary slightly between left and right ventricles. HIF-1α stabilization plays an important role in cardiac responses to HH, but not in skeletal muscle. This may be explained simply by the varying demand experienced by the two tissues. Cardiomyocytes are under constant demand for oxygen in order generate ATP to maintain contractility whereas skeletal muscle only experiences high levels of demand, or a hypoxic state, during exercise. The oxygen extraction or oxygen utilization ratio is 20-30% in skeletal muscle whereas in cardiomyocytes it is upwards of 60% (Wolff, 2007). Because of the highly oxidative capacity of cardiac muscle relative to skeletal muscle, it is logical that the heart is very sensitive to a reduction in oxygenation and experiences a higher degree of hypoxia and subsequently higher HIF expression, while skeletal muscle only experiences hypoxia during extreme exercise. Chronic HH may decrease cardiac capacity to oxidize fatty acids as seen by lower VLCAD expression in the RV, favoring HIF-1α mediated reliance on glycolytic metabolism, while the opposite effect is seen in muscle. Higher GR expression in WG follows in accordance with the rapid energy mobilization required by the fast twitch fibers comprising WG, resulting in higher WG sensitivity to the effects of GR signaling compared to PLANT (Talbot, Maves 2016). The roles of GR and HIF-1α signaling as well as their potential cross-talk in tissue responses to HH and their broader functional implications merit further investigation. Because of the presence of HIF-1α in both the LV and RV under HH in this study, in addition to the essential nature of HIF-1α in response to cardiovascular disease, further studies should look into potential utilization of HIF-1α to better cardiac patient outcomes.

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