Characterization of Molecular and Functional Changes Regulating Hypobaric Hypoxia-Induced Right Ventricular Hypertrophy

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DEDICATION

To my parents for instilling work ethic and a sense of purpose in my life

ABSTRACT

Characterization of Molecular and Functional Changes Regulating Hypoxia-Induced Right Ventricular Hypertrophy: Makhosazane Zungu, February 2007

We hypothesized that exposure to chronic hypobaric hypoxia coordinately upregulates mitochondrial regulatory genes as part of an adaptive response to sustain mitochondrial respiratory capacity and contractile function. To test this hypothesis, we initially exposed rats to 2 and 4 weeks of hypobaric hypoxia (11% O_2) and determined cardiac contractile, mitochondrial respiratory function and measured transcript levels of several mitochondrial regulators for the right and left ventricles respectively. For this part of the study, our data demonstrate the coordinate induction of several mitochondrial genes regulating mitochondrial respiratory function and increased mitochondrial DNA content in the hypertrophied RV after 2 weeks of hypoxia. These changes were not observed in the LV. These data suggest a link between the efficiency of mitochondrial oxidative phosphorylation and respiratory function to sustained RV contractile function in response to the increased load. We also found that these adaptations were generally sustained in the RV after 4 weeks of hypoxic exposure. However, the LV displayed diminished contractile and respiratory function at the 4-week time point. We suggest that this mechanism could be part of an adaptive response to conserve energy for contractile purposes.

In parallel, we explored the role of increased fatty acid utilization in the setting of right ventricular hypertrophy by employing a peroxisome proliferator-activated receptor alpha (PPARα) agonist, i.e. Wy-14,643. We found that Wy-14,643 treatment attenuated mitochondrial respiration in the RV and the LV, while promoting uncoupling of mitochondrial oxidative phosphorylation in the LV, suggesting that it may not be an ideal experimental agent to use for in vivo studies aiming to increase myocardial fatty acid oxidation rates.

We next exposed rats to 12 weeks of hypobaric hypoxia to follow up on changes observed at the 2- and 4-week time points. Furthermore, we increased cardiac FA utilization by either: a) cocoa butter, b) fenofibrate or c) coco-butter plus fenofibrate administration. Here we found sustained cardiac contractile function and diminished mitochondrial respiration in both ventricles in response to 12 weeks hypobaric hypoxia. Our data show distinct effects of cocoa butter and fenofibrate on cardiac and respiratory function in the RV and LV following hypoxic exposure. For the RV, the hypoxia-mediated increase in systolic pressure, developed pressure and RPP was reduced following fenofibrate administration under hypoxic conditions. In contrast, RV systolic and developed pressures remained relatively high following cocoa butter treatment. The cocoa butter-fenofibrate combination under hypoxic conditions largely reduced cardiac contractile function in both ventricles. Taken together, these data suggest that chronic hypobaric hypoxia triggers adaptive pathways that may reserve organisms from the potentially damaging effects of high fatty acid supply and support the concept that fuel substrate switch away from fatty acids may be an adaptive mechanism.

We next proceeded to investigate the acute and direct effects of Wy-14,643 and fenofibrate on isolated mitochondria. Our data show that acute exposure to Wy-14,643 and fenofibrate differentially perturb cardiac mitochondrial respiration, i.e. fenofibrate more potently inhibited mitochondrial respiratory function compared to Wy-14,643. We found that both electron transport chain complex I and II are inhibited by fenofibrate, while Wy-14,643 more readily inhibited complex I-dependent respiration.

SUMMARY

An imbalance between tissue oxygen supply and demand results in hypoxia. Hypoxia is caused by many factors which may range from environmental (e.g. high altitude, diving), clinical (e.g. sleep apnea, chronic obstructive pulmonary disease), low oxygen delivery (e.g. heart failure, vascular diseases, stroke, anemia), and low oxygen utilization by tissues (e.g. sepsis, metabolic myopathies). Organisms have evolved adaptive regulatory mechanisms to cope with acute and chronic exposure to hypoxia, and these include 1) pulmonary ventilation, 2) blood hemoglobin, 3) vascularity of the tissues, 4) diffusing capacity of the lungs, 5) metabolic remodeling, and 6) cellular oxygen utilization. Exposure to hypobaric hypoxia also leads to development of right ventricular (RV) hypertrophy, mainly due to an increase in RV mass secondary to pulmonary hypertension.

In the first part of the study (Chapter 2), we hypothesized that exposure to chronic hypobaric hypoxia coordinately upregulates mitochondrial regulatory genes and mitochondrial content in the hypertrophied RV, as part of an adaptive response to sustain mitochondrial respiratory capacity and contractile function. To test our hypothesis, we exposed rats to 2 and 4 weeks of hypobaric hypoxia (11% O_2) and determined cardiac contractile and mitochondrial respiratory function for the right and left ventricles, respectively. Moreover, we performed real-time quantitative RT-PCR analysis to measure transcript levels of several mitochondrial regulators. For this part of the study, our data demonstrate the coordinate induction of several genes (COXII, PGC-1α, NRF1) regulating mitochondrial respiratory function and increased mitochondrial DNA (mtDNA) content in the hypertrophied RV, linking the efficiency of mitochondrial oxidative phosphorylation and respiratory function to sustained contractile function in response to the increased load. Interestingly, these adaptations

were not observed in the LV. Moreover, these changes were generally sustained in the RV after 4 weeks of hypoxic exposure. However, at this time-point the LV began to display diminished contractile and respiratory function.

For the second part of this study, we focused on further exploring the role of increased fatty acid utilization in the setting of right ventricular cardiac hypertrophy. Cardiac hypertrophy is often associated with a substrate switch from fatty acid oxidation to glucose metabolism, due to downregulation of PPARα expression. The hypertrophied and failing heart is associated with a pronounced degree of cardiac metabolic remodeling. These changes appear to manifest in a stage-dependent manner, with earlier stages of heart failure linked to increased fatty acid utilization while end-stage failure is associated with a switch away from fatty acid metabolism (fetal switch). It is also unclear whether these fuel substrate switches are part of an adaptive process or merely an epiphenomenon. Here we proposed that PPARα activation (and subsequent increased fatty acid utilization) in the hypertrophic context would result in decreased efficiency of mitochondrial respiration, thereby leading to impaired contractile function. We devised a series of experiments to activate PPARα (using Wy-14, 643) in the hypertrophied RV (2 weeks hypoxic exposure) and to subsequently assess whether this intervention results in an adaptive or maladaptive phenotype. The major finding of this part of the study is that in vivo Wy-14,643 administration elicited direct effects on mitochondrial respiratory function and cardiac contractility. The data show that Wy-14,643 treatment attenuated mitochondrial respiration (state 3 respiration and rate of ADP phosphorylation) in the RV while promoting uncoupling of mitochondrial oxidative phosphorylation (increased state 4 respiration and basal leak) in the LV.

We then measured the effects of Wy-14,643 administration on the transcript levels of several mitochondrial regulators. The combination of Wy-14,643 and hypoxia resulted in the coordinate induction of PPARα-target genes (PDK-4 and UCP3) in the RV. Wy-14,643 treatment increased PGC-1α, NRF-1, and ANT1 levels in the right ventricle. However, this was associated with impaired respiratory capacity. The Wy-14,643-hypoxia combination increased transcript levels of PDK-4, ANT1 and decreased COXII in the LV. Also, LV, COXIV and UCP3 levels were increased, whereas PGC-1α levels were decreased in response to Wy-14,643 treatment. Reduced PGC-1α was associated with uncoupling and impaired LV function. However, the direct effects of in vivo Wy-14,643 administration make it difficult to draw firm conclusions from these data.

We next exposed rats to 12 weeks of hypobaric hypoxia (11% O_2) (Chapter 3) with two major aims: 1) To investigate whether adaptive cardiac changes we earlier observed (i.e. after 2 weeks) in the hypertrophied RV are sustained at a later time point (12 weeks), and 2) To increase cardiac fatty acid utilization in the hypertrophied heart using two approaches i) enhancing fatty acid delivery by chronic supply of a high fat diet (cocoa butter), and ii) activating PPARα using fenofibrate as a ligand. We demonstrated that cardiac contractile function is sustained (in both RV and LV) at later time points i.e. 12 weeks. Furthermore, we found that LV contractile function is now increased versus the earlier time points (2/4 weeks). Unlike the 2- and 4-week time points (Chapter 2), we observed that exposure to 12 weeks of hypobaric hypoxia resulted in diminished palmitoyl-L-carnitine oxidation and ADP phosphorylation rates (both ventricles).

Since we observed direct effects of PPARα ligands on mitochondrial respiration at longer time point (2 weeks), we investigated the direct and acute effects of two selective PPARα ligands, i.e. fenofibrate and Wy-14,643, on mitochondrial

respiratory function using rat cardiac mitochondria (Chapter 4). We also wanted to ascertain if the uncoupling effects exhibited by PPARα ligands on mitochondrial respiration are substrate-specific. Isolated left ventricular mitochondria were incubated with increasing concentrations of fenofibrate or Wy-14,643 (10, 100, 500 μM) and mitochondrial respiration determined using: malate/glutamate (complex I), succinate (complex II) and palmitoyl-L-carnitine as oxidative substrates. We found that Wy-14,643 and fenofibrate differentially inhibit mitochondrial respiratory function in isolated rat heart mitochondria. We found that acute exposure to fenofibrate markedly inhibited state 3 respiration and rate of ADP phosphorylation. Wy-14,643 elicited dose-dependent effects on respiratory capacity and phosphorylation rate. Our data suggest that both complex I and II are inhibited by fenofibrate while Wy-14,643 more readily inhibited complex I-dependent respiration

In summary, in this study we demonstrated that co-ordinate induction of mitochondrial regulatory genes in the hypertrophied RV is one of the adaptive responses to sustain mitochondrial respiratory capacity and contractile function in response to the hypoxic stimulus at earlier time points (2 and 4 weeks). Interestingly, the later time point (12 weeks) was associated with increased cardiac contractility but reduced mitochondrial respiratory capacity. We propose that this may either be an adaptive measure to conserve energy reserves, or the beginning of a maladaptive phenotype in the RV. These possibilities require further investigation.

We also found that fenofibrate treatment under hypoxia resulted in more severe effects on contractile function, particularly in the LV. Furthermore, we found that adaptive mitochondrial pathways and function of the hypertrophied RV triggered in response to hypobaric hypoxia were not blunted in the presence of high fat supply (cocoa butter). These data therefore indicate that chronic hypobaric hypoxia triggers adaptive pathways that may rescue organisms from the potentially damaging effects

of higher fatty acid supply. My thesis also extends previous work by providing a potential mechanism whereby PPARα activation, and by implication increased fatty acid oxidation, may impair contractile function of the hypertrophied heart i.e. by uncoupling mitochondrial oxidative phosphorylation. Furthermore, the data support the concept that the fuel substrate switch away from fatty acids in the hypertrophied heart may be an adaptive mechanism.

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Chapter 1

General Introduction

An adequate supply of oxygen is vital for the survival of all living organisms. Hypoxia is defined as a relative shortage of oxygen due to lowered partial pressure of oxygen within the cellular environment [Schmedtje and Ji, 1998; Muertz, 1965]. Since a constant oxygen supply is essential to sustain life, organisms have evolved sophisticated molecular sensing mechanisms in order to respond and adapt to lowered oxygen levels (hypoxia). I will now review some of the major adaptive responses to chronic hypoxic exposure, with particular emphasis on the heart.

1. Adaptive measures in response to hypoxia

1.1. Oxygen sensing

There are multiple oxygen sensing mechanisms proposed to relay lowered oxygen availability to the intracellular machinery. These include the NAD(P)H oxidase enzyme family, components of the mitochondrial electron transport chain (complex III or IV) [Acker et al., 2006; Murdoch et al., 2006], reactive oxygen species (ROS) [Shah and Sauer, 2006; Chandel and Schumacker, 2000; Michiels et al., 2002], and nitric oxide (NO) [Mateo et al., 2003; Hagen et al., 2003]. An important physiological response to hypoxia requires the coupling of mitochondrial oxygen sensors with downstream signaling molecules in order to activate adaptive functional responses [Goldenthal and Marin-Garcia, 2004]. Hypoxia inducible factors (HIFs) have been identified as key mediators relaying reduced oxygen levels to intracellular signaling and transcriptional pathways. Activated HIF-1 comprises two subunits i.e. HIF-1α and HIF-1β [Wang et al., 1995], with the former identified as a key transcription factor regulating oxygen-dependent gene transcription. The HIF-1α transcriptional system senses oxygen availability to subsequently induce target genes, thereby orchestrating adaptive responses such as increased glycolysis, angiogenesis,

erythropoiesis [Semenza, 2000; Semenza, 2004], and hypoxia-induced pulmonary hypertension [Yu et al., 1999; Shimoda et al., 2001]. HIF-1α is constitutively expressed in the heart and several other tissues [Semenza, 1998]. Under conditions of normal oxygen supply HIF-1α undergoes prolyl-hydroxylation by specific cellular prolyl hydroxylase domain-containing enzymes (PHDs) [Semenza, 2001], and subsequent ubiquitination and degradation by the 26S proteasome (Figure 1) [Huang et al., 1998; Kallio et al., 1999; Salceda and Caro, 1997].

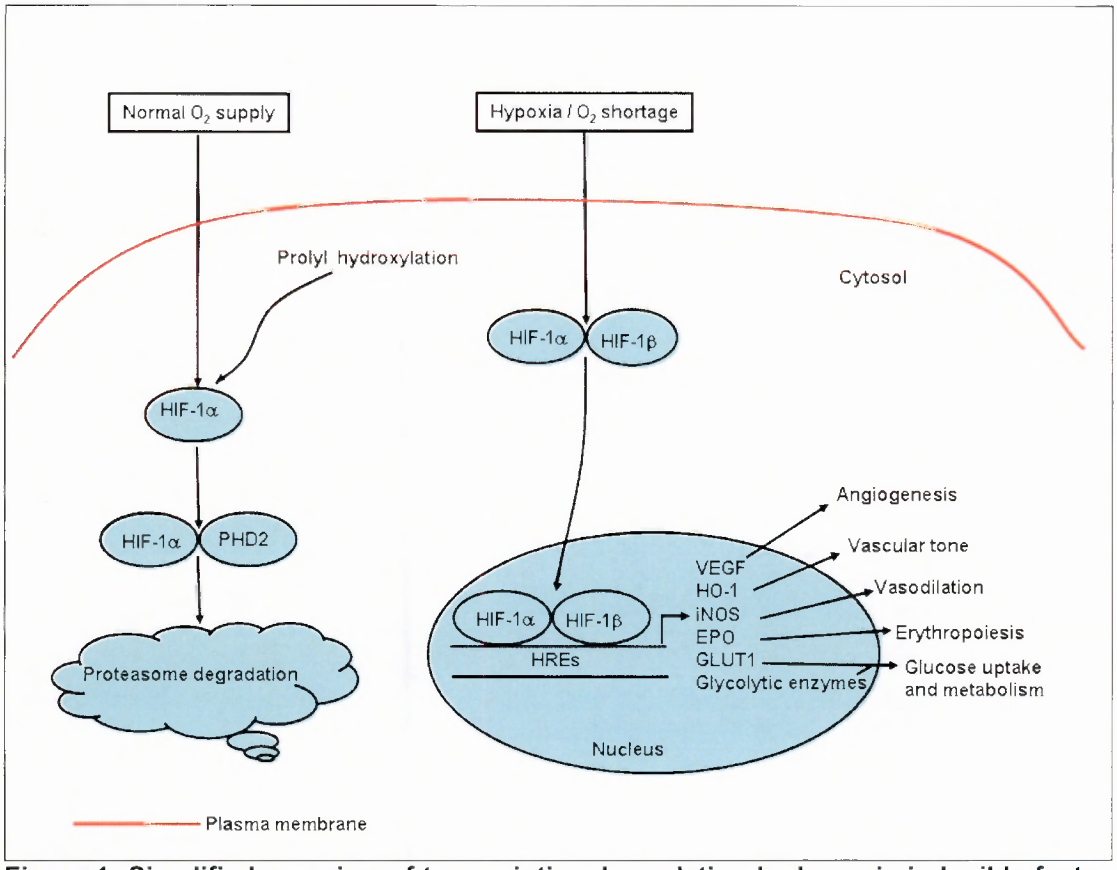

Figure 1: Simplified overview of transcriptional regulation by hypoxia inducible factor-1α (HIF-1α) under normoxic and hypoxic conditions. Abbreviations: hypoxia-inducible factor-1β (HIF-1β), prolyl-4-hydroxylase-2 (PHD2), hypoxia response element (HRE), vascular endothelial growth factor (VEGF), heme oxygenase-1 (HO-1), inducible nitric oxide synthase (iNOS), erythropoietin (EPO), glucose transporter 1 (GLUT1).

Expression of the PHDs varies among cell types and in response to physiologic stimuli [Metzen et al., 2003; D'Angelo et al., 2003]. Prolyl-4-hydroxylase-2 (PHD2) is ubiquitously expressed and exhibits the highest specificity toward HIF-1α [Hirsila et al., 2003]. However, under hypoxic conditions HIF-1α stabilizes thus allowing it to

dimerize with its obligate partner, HIF-1β, to alter the expression of target genes. HIF-1β is also referred to as aryl hydrocarbon nuclear translocase (ARNT). HIF-1α binds to specific hypoxia response elements (HREs) i.e. 5′ -ACCTGC- 3′ that is usually located within, or adjacent to target genes in the 5′-or 3′-flanking sequences [Wang et al., 1995; Tian et al., 1997; Wiesener et al., 1998].

Since ARNT is generally abundant, availability of HIF-1α is the rate limiting step in the formation of HIF-ARNT dimers. As indicated before, HIF-1α plays a crucial role as transcription regulator of several adaptive genes to counteract reduced oxygen supply (Figure 1). For example, vascular endothelial growth factor (VEGF) [Forsythe et al., 1996] is known to be induced by hypoxia in vascular endothelial cells [Namiki et al., 1995]. Moreover, increased VEGF expression leads to angiogenesis in hypoxic tissues [Shweiki et al., 1992] in order to enhance oxygen delivery. There are conflicting reports regarding the development of new myocardial capillaries in animals exposed to hypoxia. While some [Rotta, 1943; Clark and Smith, 1978] have reported decreased ventricular capillary density in chronically hypoxic guinea pigs and rats, others [Miller and Hale; 1970] have found increased capillary density (right and left ventricles) or no changes in the left ventricle [Moravec et al., 1983]. Nevertheless, coronary angiogenesis is considered by many to be a compensatory mechanism to increase oxygen delivery to the heart [Zarain-Herzberg et al., 1996].

HIF-1α also induces the gene expression of erythropoietin (EPO) [Semenza and Wang, 1992], heme oxygenase-1 (HO-1) [Dawn and Bolli, 2005], inducible nitric oxide synthase (iNOS) [Jung et al., 2000], glucose transporter (GLUT1) [Loike et al., 1992], and a panel of glycolytic enzymes such as glyceraldehyde-phosphate dehydrogenase (GAPDH) [Semenza, 1999; Hoppeler et al., 2003], phosphoglycerate kinase 1 (PGK1), aldolase A, hexokinase (HK), and lactate dehydrogenase A [Iyer et

al., 1998; Ryan et al, 1998; Semenza et al., 1994, 1996]. These adaptive measures are not only an attempt to increase oxygen delivery to the heart but also to switch to increased utilization of an alternate metabolic fuel substrate i.e. glucose, that is more efficient in terms of ATP per mole of O_2 (compared to fatty acids) when oxygen supply is limiting. The metabolsim of glucose in mammalian heart is 25-50% more oxygen efficient than the metabolism of free fatty acids [Holden et al., 1995].

Although HIF-1 is a pivotal transcriptional modulator activated in response to diminished oxygen supply, other transcriptional regulators also play important roles in this process. For example, the Sp family (Sp1 and Sp3) of transcription factors have been implicated in hypoxia-sensitive gene expression, acting via GC-rich promoter elements [Boulikas, 1994]. Sp1 is a transcriptional activator while Sp3 may act either as a repressor or activator, depending on the promoter and cellular context [Dennig et al., 1996; Birnbaum et al., 1995; Discher et al., 1998]. Other oxygen-sensitive transcriptional regulators that are directly oxygen-sensitive and not under control of HIF-1 include, for example, the cAMP response element-binding protein-1 (CREB-1) [Safran and Kaelin, 2003], activator protein-1 (AP-1), nuclear factor-κB (NF-κB) [Pahl and Baeurle, 1994; Michiels et al., 2002], peroxisome proliferator-activated receptor α (PPARα), retinoid X receptor α (RXRα) [Sharma et al., 2004; Razeghi et al., 2001b; Huss et al., 2001], and Nkx2.5 [Bar et al., 2003; Pahl and Baeuerle, 1994].

1.2. Hypoxia-mediated cardiac protection

The physiologic responses to chronic hypoxia include polycythemia, pulmonary hypertension, and angiogenesis [Chien et al., 1993; Hasenfuss et al., 1994; Zarain-Herzberg et al., 1996]. Several studies have confirmed such responses in laboratory animals exposed to low oxygen levels [Feldman et al., 1993; O'Rourke et al., 1999;

Studer et al., 1994; Flesch et al., 1996; Litwin et al., 1997; Sipido et al., 2000]. Furthermore, chronic hypoxia also causes an increase in hematocrit [Hunter et al., 1974] as an adaptive response to enhance oxygen delivery. An increase in haemoglobin and red cell count enhances the blood oxygen carrying capacity despite lower than normal arterial oxygen saturation [Norese et al., 2002].

Adaptation to chronic hypoxia is characterized by a variety of functional changes that may protect the heart during episodes of enhanced work. For example, previous studies found that the incidence of myocardial infarction is lower in people naturally acclimatized to high altitude (e.g. Peru, 4000 m) [Hurtado, 1960; Monge et al., 1990]. Moreover, Meerson et al. (1973) showed that rats exposed to chronic intermittent hypoxia and exposed to hypoxia for only a portion of each 24 hr period develop smaller myocardial infarcts. In agreement, Opie et al. (1978) found that exposure to high altitude hypoxia protects against coronary occlusion. Longer term studies show similar results. For example, pre-exposure to chronic hypoxia in adult rat hearts conferred subsequent resistance against an acute hypoxic injury i.e. four months after removal from the hypoxic environment [Ostadal et al., 1995]. This long-lasting effect of hypoxia is in contrast to the protection triggered by ischemic preconditioning, which usually persists for only 3-4 days [Fitzpatrick et al., 2005] and therefore offers the possibility of novel therapeutic intervention. The intracellular signaling mechanisms directing hypoxia-mediated cardiac protection are numerous and the subject of ongoing investigations For instance, adaptation to chronic myocardial hypoxia results in increased resistance to ischemia [Baker et al., 1995] associated with enhanced nitric oxide production from the NOS3 isoform of nitric oxide synthase (NOS) [Shi et al., 2000]. Furthermore, it has been shown that activation of NOS3 activates the sarcolemmal ATP-sensitive potassium (sarcK_{ATP}) channels via a cGMP-dependent mechanism under normoxic and hypoxic conditions [Baker et al., 2001].

Since the mitochondrial ATP-sensitive potassium (mitoK$_{ATP}$) channel is thought to be a key regulator of cardioprotection [Gross and Fryer, 1999], chronic hypoxia may be a novel way to activate this process.

1.3. Chronic hypoxia induces pulmonary hypertension and right ventricular hypertrophy

It is well documented that chronic exposure to high altitude hypoxia results in pulmonary hypertension [Rabinovitch et al., 1979] via vasoconstriction of the pulmonary arteries. The development of pulmonary hypertension is a highly complex process involving several intracellular pathways [Humbert et al., 2004]. In rats exposed to hypobaric hypoxia, hypoxic pulmonary hypertension develops during the first two weeks of exposure and then stabilizes, and does not increase in severity during the adaptation phase [Herget et al., 1978; Reeves and Herget, 1984]. Hypoxia-induced polycythemia is another factor that contributes to pulmonary hypertension by increasing blood viscosity [Naeye, 1961; Janssens et al., 1991; Barer et al., 1983; Fedde and Wideman, 1996]. This subsequently increases the load on the right ventricle (RV), ultimately leading to the selective development of RV cardiac hypertrophy (RVH) in animals and humans [Ostadal et al., 1995; Kolar and Ostadal, 1991; Favret et al., 2001; Morel et al., 2003; Penaloza et al., 1963; Rabinovitch et al., 1979; Reeves and Herget, 1984]. However, Hislop and Reid (1976) demonstrated that the morphological changes in chronic hypoxia-induced pulmonary hypertension were reversible, requiring ~8 weeks to return to normal.

Several studies have simulated this condition by employing experimental protocols whereby animals are exposed to chronic hypobaric hypoxia. Only a handful of animal models exist that are able to induce selective right ventricular hypertrophy without left

ventricular growth. These include experimental pulmonary artery stenosis [Zierhut et al., 1990; Adachi et al., 1991; Ikeda et al., 1990; Olivetti et al., 1988; Zimmer, 1992], monocrotaline-treatment [Chen et al., 2001; Brown et al., 1998], and pulmonary hypertension by lung irradiation [Zimmer and Zierhut, 1991]. Although the left ventricular mass is not significantly changed by exposure to hypoxia [Rabinovitch et al., 1979; Ostadal et al., 1995; Kolar and Ostadal, 1991], it is recognized that there is a relationship between the functioning of the two ventricles. Therefore, impairment of the RV may eventually influence left ventricular (LV) function (or *vice versa*) [Clyne et al., 1989; Dittrich et al., 1992; Hill et al., 1997; Mizushige et al., 1989; Yu et al., 1996].

1.4. Hypoxia induces mitochondrial biogenesis

Another adaptation to chronic hypoxia is the proliferation of mitochondria. Previous studies in cardiac tissues have reported increased number of smaller mitochondria in rat hearts exposed to hypobaric hypoxia for 21 days [Nouette-Gaulain et al., 2005; Costa et al., 1988]. This is proposed to be an adaptive response to increase mitochondrial surface area, and thus bioenergetic capacity. The basic features of the process of mitochondrial biogenesis will now be summarized.

The ability to modulate mitochondrial number and function is an important adaptive response in all eukaryotes [Wu et al., 1999]. The heart is a highly oxidative tissue that produces more than 90% of its energy from mitochondrial respiration [Ventura-Clapier et al., 2004]. In the heart, mitochondria occupy ~40% of cardiomyocyte space, arranged in rows between myofilaments and under the sarcolemma [Barth et al., 1992]. In addition to energy production, mitochondria are major regulators of apoptosis and therefore play a key role in determining life and death of the mammalian cell [Lee et al., 2000]. Mitochondrial biogenesis increases during cardiac

hypertrophy and exercise and following treatment with xenobiotics, thyroxin, glucocorticoids and electrical stimulation [Attardi et., 1977; Hood, 2001; Totland et al., 2000; Lundgren et al., 1987; Weber et al., 2002; Williams et al., 1987; Nelson, 1990; Xia et al., 1997].

Mitochondria divide during mitosis, providing daughter cells with a normal complement of mitochondria [Bogenhagen and Clayton, 1977]. In agreement, Sanger et al. (2000) showed that mitochondrial mass increases from the onset of S-phase through M-phase. Besides mitosis, there is a suggestion that mitochondria can be either synthesized from submicroscopic precursors present in the cytoplasm, bud from other membranous structures of the cell, or grow and divide from pre-existing mitochondria [Clementi and Nisoli, 2005]. Mitochondrial divisions are not always linked to the cell cycle. For example, muscle mitochondria proliferate during myogenesis and in response to exercise [Brunk, 1981; Moyes et al., 1997]. Mitochondrial biogenesis is accompanied by plasticity of the mitochondrial external shape which is arranged in extended tubular networks [Chen, 1988]. Continuous physical interaction of mitochondria through fusion ensures exchange of both membrane and matrix components to restore local depletions and maintain mitochondrial function [Nakada et al., 2001]. Furthermore, mitochondria have been shown to import most of their phospholipids from the cytoplasm to form and preserve membrane integrity [Moyes and Hood, 2003].

Mitochondrial biogenesis depends on the coordinated expression of both the nuclear and mitochondrial genomes. Animal mitochondria possess their own double-stranded circular DNA (mtDNA) that encodes 13 subunits of the oxidative phosphorylation system (OXPHOS) [Anderson et al., 1991]. Nuclear genes encode all the proteins and enzymes involved in mtDNA replication, transcription, and translation in mitochondria [Shadel and Clayton, 1997]. Two transcription factors play a key role in

facilitating interaction between the nucleus and the mitochondrion. Here nuclear respiratory factors (NRFs) bind and activate the promoters of various nuclear genes that encode for components of mitochondrial oxidative phosphorylation (OXPHOS), and the mitochondrial transcription factor A (mtTFA) that regulate mtDNA replication and transcription [Scarpulla, 2002a]. Upon stimulation, mtTFA translocates to the mitochondrion, where it stimulates mitochondrial biogenesis as indicated by increased mitochondrial DNA replication and mitochondrial gene expression [Garesse and Valejo, 2001; Larsson et al., 1998] (Figure 2).

Peroxisome proliferator-activated receptor–gamma coactivator-1α (PGC-1α) is a key nuclear receptor co-activator for mitochondrial biogenesis and controls mitochondrial number and function in response to energy demands through its interaction with NRFs [Lehman and Kelly, 2002; Scarpulla, 2002a,b; Lehman et al., 2000; Puigserver and Spiegelman, 2003]. In addition, the NRFs (NRF-1 and NRF-2) transcription factors mediate the expression of a number of nuclear genes involved in mitochondrial OXPHOS including subunits of cytochrome c oxidase (COX) and ATP synthase [Vega, 2000; Scarpulla, 2002a,b]. PGC-1α also coactivates estrogen-related receptor α (ERRα), a member of the orphan nuclear receptor family [Giguere et al., 1988; Heard et al., 2000; Hong et al., 1999]. In turn, ERRα interacts with NRF-1 and NRF-2 to regulate mitochondrial biogenesis and expression of OXPHOS enzyme genes [Mootha et al., 2004; Schreiber et al., 2004].

PGC-1α and PGC-1β play key roles in controlling mitochondrial metabolism and oxidative phosphorylation, and in activating expression of nuclear-encoded mitochondrial genes [Scarpulla, 2002b]. In agreement with this, overexpression of PGC-1 α in transgenic mice leads to mitochondrial proliferation in the heart [Lehman et al., 2000], adipocytes [Lowell and Spiegelman, 2000], and myoblasts [Wu et al.,

1999]. Both isoforms are highly expressed in tissues with high oxidative capacity including heart, skeletal muscle, brown adipose tissue, and liver (Puigserver et al., 1998).

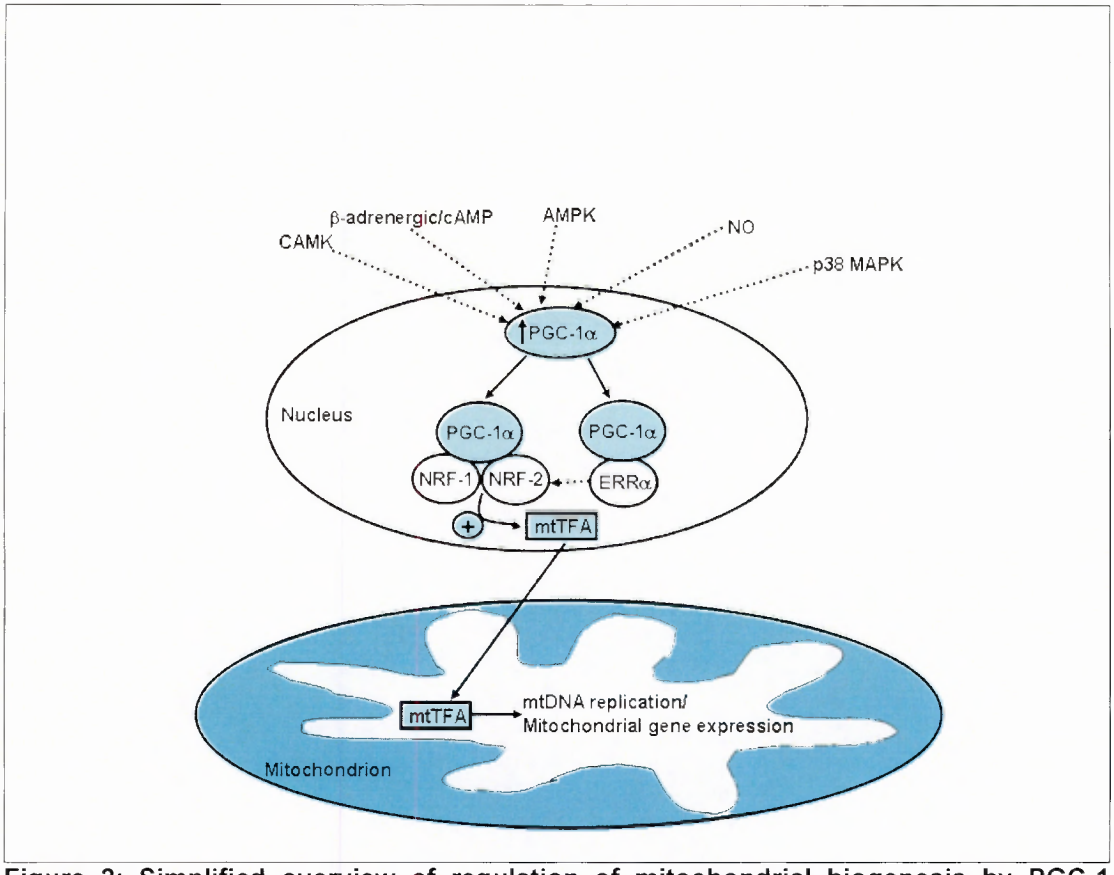

Figure 2: Simplified overview of regulation of mitochondrial biogenesis by PGC-1. Abbreviations: Ca^{+2}-calmodulin kinase (CAMK), cyclic adenosine monophosphate (cAMP), AMP kinase (AMPK), nitric oxide (NO), p38 mitogen-activated protein kinase (p38MAPK), peroxisome proliferator-activated receptor-gamma coactivator-1 α (PGC-1α), nuclear respiratory factor-1 and -2 (NRF-1, NRF-2), estrogen–related receptor α (ERRα), mitochondrial transcription factor (mtTFA), mitochondrial DNA (mtDNA).

PGC-1α activity and expression levels are induced by physiological stimuli such as cold exposure, fasting, and exercise, stimuli known to promote fatty acid metabolism [Lehman et al.; 2000; Goto et al., 2000; Baar et al., 2002]. Signaling pathways involved in the regulation of mitochondrial biogenesis, and therefore activation of PGC-1α, include p38 mitogen-activated protein kinase (p38MAPK) [Puigserver et al., 2001], β-adrenergic/cAMP [Nisoli et al., 2003], nitric oxide [Nisoli et al., 2003], AMP kinase [Zong et al., 2002], and Ca^{+2}-calmodulin kinase (CAMK) [Wu et al., 2002].

1.5. Metabolic adaptation in response to hypoxia

Cardiac metabolic remodeling is another major adaptation during exposure to hypobaric hypoxia. Here, the primary adaptation is a fuel substrate switch from fatty acids to glucose, since the latter is proposed to be a more energetically-efficient fuel substrate when oxygen supply is limiting [Young et al., 2001a; Razeghi et al., 2001a; Barger and Kelly, 1999; Sack et al., 1997]. Likewise, a similar fuel substrate switch has been observed in the hypertrophied heart [Barger and Kelly, 1999; Bishop and Altschuld, 1970; Taegtmeyer and Overturf, 1988; Christe and Rodgers, 1994; Takeyama et al., 1995; Buttrick et al., 1994; Feinendegen et al., 1995]. Since the focus of my thesis relates to metabolic adaptations of the heart in response to hypobaric hypoxia, I will now summarize basic aspects of cardiac metabolism followed by a discussion of conditions where myocardial fuel substrate utilization is altered, particularly in response to chronic oxygen lack.

1.5.1. Cardiac glucose metabolism

Pioneering studies have shown that glucose, lactate and fatty acids are the major fuel substrates of the mammalian heart [Bing et al, 1953, 1954]. Fatty acids are metabolized in mitochondrial matrix by β-oxidation and account for ~60 – 90 % of the total energy production in the normal adult mammalian heart [van der Vusse et al., 1992]. Glucose is catabolized by glycolysis and glucose oxidation, accounting for ~10–40 % of total cardiac energy production [Gertz et al., 1988]. However, despite varying degrees in substrate preference, it is thought that the heart functions optimally when oxidizing both fuel substrates simultaneously.

The heart obtains carbohydrates for metabolism either from the uptake of glucose or lactate, or by the breakdown of glycogen. Glucose uptake into the heart is facilitated by two glucose transporter isoforms, GLUT1 and GLUT4 [Gould and Holman, 1993], a rate-limiting step for exogenous glucose utilization [Depre et al., 1999]. Expression of glucose transporters is developmentally regulated with GLUT1 highly expressed during the fetal stages of development, whereas GLUT4 is postnatally induced and an insulin-sensitive isoform [Santalucia et al., 1992]. GLUT1 is a major mediator of basal cardiac glucose uptake and normally accounts for ~30 % of the total cardiac glucose transporters in the adult heart [Laybutt et al., 1997; Fischer et al., 1997]. The number of glucose transporters and the transmembrane glucose gradient regulate glucose transport into the myocardium [Kodde et al., 2006]. In response to particular stimuli, for example, insulin, ischemia or increased work, there is a higher demand for glucose utilization. In response, GLUTs are mobilized from intracellular vesicular stores to migrate to the sarcolemma, thereby increasing myocardial glucose uptake [Slot et al., 1991; Uphues et al., 1994].

After uptake, glucose is either converted to glycogen or enters the glycolytic pathway where it is ultimately converted to pyruvate with the production of two ATP molecules (Figure 3). Upon entering the cell, free glucose is rapidly phosphorylated by hexokinase to form glucose-6-phosphate (G6P). This ensures that the concentration gradient across the plasma membrane is maintained and that glucose does not leak back out of the cell. G6P can either be converted to glycogen for storage (reaction catalyzed by glycogen synthase) or converted to fructose-6-phosphate (F6P) by the action of phosphohexose isomerase. Glycogen can be mobilized to re-enter the glycolytic pathway via glycogenolysis (reaction catalyzed by glycogen phosphorylase). Phosphofructokinase-1 (PFK-1) is a key regulatory enzyme in the glycolytic pathway and catalyzes the first irreversible step in the breakdown of F6P to

fructose 1,6-bisphosphate (F1,6-BP) (Hue et al., 2002). PFK-1 is inhibited by ATP and activated by ADP, AMP, and P_i [Pogson and Randle, 1966].

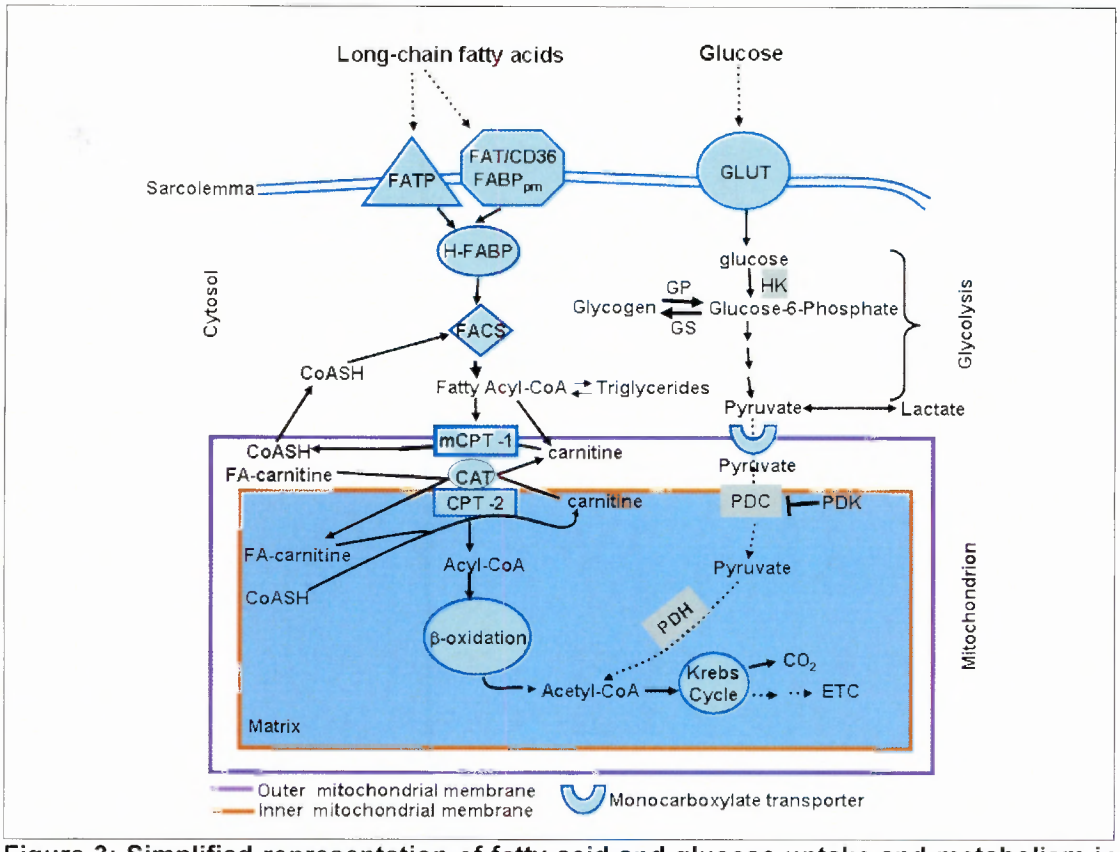

Figure 3: Simplified representation of fatty acid and glucose uptake and metabolism in the normal adult myocardium. Abbreviations: fatty acid transport protein (FATP), fatty acid translocator (FAT/CD36), heart-type fatty acid binding protein (H-FABP), plasma membrane fatty acid binding protein ($FABP_{pm}$), fatty acyl-CoA synthetase (FACS), muscle-type carnitine palmitoyl transferase-1 (mCPT-1), carnitine palmitoyl transferase-2 (CPT-2), carnitine acyl transferase (CAT), coenzyme A (CoASH), GLUT (glucose transporter), hexokinase (HK), pyruvate dehydrogenase kinase (PDK), pyruvate dehydrogenase complex (PDC), pyruvate dehydrogenase (PDH), electron transport chain (ETC).

Fructose 1,6-bisphosphate is subsequently converted to glyceraldehyde 3-phosphate, a reversible reaction catalyzed by fructose 1, 6- bisphosphate aldolase. Further downstream, glyceraldehyde-3-phosphate dehydrogenase catalyzes the conversion of glyceraldehyde 3-phosphate to 1, 3-disphophoglycerate, producing NADH in the process. Sequential reactions catalyzed by phosphoglycerate kinase, phosphoglyceromutase and enolase ultimately result in the production of 2x phosphoenolpyruvate (PEP), which is in turn converted by pyruvate kinase to 2x pyruvate.