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Prenatal Hypoxia Reduces Mitochondrial Protein Levels and Cytochrome c Oxidase Activity in Offspring Guinea Pig Hearts

Yazan M. Al-Hasan, PhD[1,2], Gerard A. Pinkas, BS[1,2], and Loren P. Thompson, PhD[1,2]

Abstract

Prenatal hypoxia (HPX) reduces mitochondrial cytochrome c oxidase (CCO and COX) activity in fetal guinea pig (GP) hearts. The aim of this study was to quantify the lasting effects of chronic prenatal HPX on cardiac mitochondrial enzyme activity and protein expression in offspring hearts. Pregnant GPs were exposed to either normoxia (NMX) or HPX (10.5%O_2) during the last 14 days of pregnancy. Both NMX and HPX fetuses, delivered vaginally, were housed under NMX conditions until 90 days of age. Total RNA and mitochondrial fractions were isolated from hearts of anesthetized NMX and HPX offspring and showed decreased levels of CCO but not medium-chain acyl dehydrogenase activity, protein levels of nuclear- and mitochondrial-encoded COX4 and COX1, respectively, and messenger RNA expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha, COX5b, and 4.1 compared to NMX controls. Prenatal HPX may alter mitochondrial function in the offspring by disrupting protein expression associated with the respiratory chain.

Keywords

cardiac, electron transport chain, programming

Introduction

Chronic intrauterine hypoxia (HPX) induces fetal growth restriction and contributes to fetal programming.[1-4] Fetal HPX has been shown to alter signaling mechanisms of multiple fetal organs such as the heart,[5-9] brain,[10,11] liver,[12-16] and skeletal muscle.[16] Chronic intrauterine HPX has been shown to contribute to permanent changes in cardiovascular function of the offspring through programming.[9,13,17-19] Central to all organ functions is the role of mitochondria in the cell's ability to maintain adequate energy supply in the presence of low oxygen levels. Yet, the postnatal effect of intrauterine HPX on cardiac mitochondrial function in the offspring remains incompletely understood.

The embryonic heart relies on anaerobic glycolysis, lactate oxidation, and fatty acid metabolism for generating adenosine triphosphate (ATP).[20] The immature heart utilizes glycolysis as its predominant energy pathway because of the availability of glucose in utero and the upregulation of enzymes in the glycolytic pathway.[21] As the fetal heart matures, fatty acid oxidation is also utilized in cellular respiration for ATP synthesis but to a smaller percentage compared to glycolysis.[20] At the time of birth, the immature heart undergoes a metabolic switch, increasing its reliance on fatty acid oxidation as circulating free fatty acids increase and the dependence on the glycolytic pathway is decreased due to downregulation of glycolytic enzymes and negative feedback regulation of glycolysis.[20,21] Since the heart has a high metabolic rate, its reliance on mitochondrial function for energy production is critical for normal cardiac function.[22]

Proper regulation of mitochondrial respiration is important for maintaining an efficient energy supply for the heart. This is met by the synthesis of ATP by F_1F_0ATPsynthase, which is dependent on the electron flux through the electron transport chain, the resulting H+ gradient across the inner mitochondrial membrane by the electron transport chain, and the adenosine diphosphate levels. Cytochrome c oxidase (CCO) is the major site of O_2 consumption in the mitochondria and the terminal site of reduction of O_2 to H_2O. Altered regulation of the mitochondrial respiratory chain can result in inefficient respiration where ATP synthesis is reduced.[23] In adult rat hearts exposed to hypobaric HPX of 11%O_2 for 7 days, left ventricles showed a decrease in mitochondrial transcript levels of peroxisome

[1] Department of Physiology (YMA), University of Maryland, Baltimore, MD, USA
[2] Department of Obstetrics, Gynecology and Reproductive Sciences (GAP, LPT), University of Maryland, Baltimore, MD, USA

Corresponding Author:
Loren P. Thompson, Department of Obstetrics, Gynecology and Reproductive Sciences, 655W Baltimore St, Bressler Research Building, Rm 11-029, Baltimore, MD 21201, USA.
Email: lthompson1@umm.edu

proliferator–activated receptor gamma coactivator 1-alpha (PGC-1α), cytochrome c oxidase subunit II (COXII), and uncoupling proteins, which was associated with a decrease in mitochondrial respiratory activity.[22] Similarly, a 21-day exposure to chronic HPX also decreased the activities of complexes I, II, III, and IV in both adult heart ventricles.[24] In addition, adult rat hearts exposed to HPX followed by normoxia (NMX) had decreased complex III activity and postischemic contractile dysfunction.[25] Further, cardiac mitochondrial energy metabolism was not fully restored after NMX recovery from chronic HPX, suggesting a permanent alteration in mitochondrial respiration to HPX stress.[26]

The role of prenatal HPX on fetal cardiac mitochondrial function has had limited attention. We have reported that chronic maternal HPX of pregnant guinea pigs generates increases in fetal cardiac HPX-inducible factor (HIF-1α),[6] peroxynitrite,[7] and malondialdehyde levels,[27] indicative of HPX stress. Further, we have shown that chronic HPX generates oxidative and nitrosative stress within fetal heart ventricles,[7] concomitant with reduced cardiac CCO activity.[27] Therefore, chronic HPX has a negative impact on fetal cardiac mitochondrial function although its lasting consequences on cardiac function in the offspring remain unknown. Only 2 studies have investigated the role of mitochondrial function in programming of offspring hearts, with one showing decreased rat cardiac CCO activity with maternal copper-deficient diet[28] and the other increased sheep cardiac mitochondrial H_2O_2 release with maternal dexamethasone administration.[29] Given the impact of intrauterine stress on cardiovascular programming and cardiac contractile function of the offspring,[17,18,30] we propose that chronic *in utero* exposure to HPX alters the expression of mitochondrial proteins that render the offspring hearts susceptible to injury. We hypothesize that the HPX-induced decrease in CCO activity measured in fetal guinea pig hearts[27] increases the vulnerability of the offspring heart by compromising mitochondrial function. This was tested by measuring the enzyme activities of medium-chain acyl-coenzyme A dehydrogenase (MCAD) and CCO, messenger RNA (mRNA)/protein expression of CCO subunits, and PGC-1α and NRF-1, key transcription factors important in the regulation of gene expression associated with mitochondrial function[31] in guinea pig hearts of offspring exposed to HPX in utero.

Methods

Animal Model

Female Duncan-Hartley guinea pigs (term = 65 days) were purchased from a commercial breeder (Elm Hill Breeding Labs, Chelmsford, Massachusetts) and time mated. Pregnant guinea pig sows were placed in a plexiglass chamber containing 10.5% O_2 for the last 14 days of pregnancy and allowed to deliver. Normoxic controls were maintained at room air (21%O_2) throughout their pregnancy. In a separate group of pregnant animals, maternal percentage O_2 saturation (SpO_2) had been previously measured using a STARR MouseOx pulse oximeter (STARR Life Sciences, Inc, Torrington, Connecticut) on the foot pad of animals quantifying a significant decrease in arterial blood oxygenation of HPX versus NMX animals (maternal SpO_2 values: 66.1% ± 1.4% vs 97.7% ± 0.2% [$P < .05$]; HPX [n = 10] vs NMX [n = 10] animals). In addition, this generates HPX fetuses that exhibit increased levels of fetal cardiac HIF-1α protein,[6] hepatic hypoxyprobe staining[8] as well as growth restriction.[27] After birth, both NMX and HPX newborn pups were reared with their mothers, weaned at 30 days of age, and housed in room air with access to ad libitum food and water until 90 days. Male offspring guinea pigs were studied at an age (90 days old) of reproductive maturity and at the plateau phase of the growth curve. Sex differences were not studied. Both NMX and HPX offspring were anesthetized with ketamine (80 mg/kg intraperitoneal [ip]) and xylazine (1 mg/kg ip), and lidocaine was given subcutaneously along the midline of the abdomen prior to dissection. Arterial blood pressures were measured from the right brachial artery cannulated in anesthetized animals. Body weights, heart weights, and heart weight–body weight ratios were measured and left and right ventricles were excised and snap frozen in liquid N_2 and stored at −80°C. The methods used were approved by the University of Maryland Animal Care Committee and conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Mitochondrial Isolation

Enriched tissue mitochondrial fractions were generated in a similar manner as described previously.[32] Briefly, frozen left and right cardiac ventricles of prenatally NMX and HPX offspring hearts were pulverized with mortar and pestle and 30 to 50 mg homogenized in ice-cold mitochondrial isolation buffer (250 mmol/L sucrose, 5 mmol/L HEPES, 1 mmol/L EDTA, pH 7.5) in a Next Advance Bullet Blender (Averill Park, NY) for 5 minutes and centrifuged at 4°C at 500*g* to remove the cellular debris. The supernatant fractions were centrifuged at high speed (12.5 × 1000*g*) to generate an enriched mitochondrial pellet fraction. Cardiac mitochondrial fractions were resuspended in homogenizing buffer and protein concentrations were determined by the Bradford Protein Assay (Bio-Rad Laboratories, Hercules, California).

Cytochrome c Oxidase Assay

Cytochrome c oxidase is the terminal complex of the electron transport chain and the main site of O_2 consumption. The CCO activity of the left and right ventricles of NMX and HPX offspring hearts was quantified by monitoring the oxidation rate of reduced cytochrome C. Equal amounts of isolated mitochondria (0.5 μg) from NMX and HPX offspring heart ventricles were solubilized with 50 μmol/L n-dodecyl-beta-D-Maltoside and added to a 96-well plate containing the assay buffer (100 mmol/L $K^+PO_4^-$ pH 7.4 and 220 μmol/L reduced cytochrome c). Samples were read at 550 nm and the activity values expressed as μmol/L of oxidized cytochrome c/min/mg of mitochondria.

Medium-Chain Acyl-CoA Dehydrogenase Assay

Medium-chain acyl-CoA dehydrogenase is a mitochondrial enzyme that oxidizes medium-chain (6-10 carbon) fatty acids and reduces flavin adenine dinucleotide (FAD) to $FADH_2$. The MCAD activity of left and right ventricles of NMX and HPX offspring hearts was quantified by monitoring the rate of ferrocenium reduction to ferrocene. Briefly, equal amounts (20 μg) of solubilized mitochondria of NMX and HPX offspring heart ventricles were added to a buffered solution containing 100 mmol/L $K^+PO_4^-$ pH 7.4, 1 mmol/L EDTA, 500 μmol/L sodium tetrathionate, and 200 μmol/L ferrocenium hexafluorophosphate in a 96-well plate. The reaction was initiated by the addition of 500 μmol/L octanoyl-CoA, the 8-carbon substrate for MCAD, and read at 300 nm. The MCAD activity is expressed as mmol/L reduced ferrocenium/min/mg of mitochondria.

Mitochondrial Protein Western Blot

Left ventricles were isolated from NMX and HPX offspring hearts and the protein expression of COX4, a nuclear-encoded subunit of CCO, and COX1, a mitochondria-encoded subunit of CCO, was analyzed using Western blot analysis. Briefly, 5 μg of mitochondrial protein extracted from offspring NMX and HPX left ventricles were incubated with Laemmli Buffer at 55°C for 10 minutes, resolved on a 4% to 15% precast gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to an Immun-Blot polyvinyl difluoride membrane (Bio-Rad). The membranes were probed with a primary mouse monoclonal antibody (1:10 000) that recognizes COX4 and COX1 (Mitosciences, Eugene, Oregon) in 5% nonfat dry milk over night at 4°C. Membranes were then incubated for 1 hour in 5% nonfat dry milk containing horseradish peroxidase-conjugated chicken anti-mouse secondary antibody (1:10 000, Santa Cruz Biotechnology, Santa Cruz, California). Bands were detected and visualized using enhanced chemiluminiscence (Western Lightning Plus, Perkin Elmer, Waltham, Massachusetts) and quantified using densitometry in ImageJ (U.S. National Institutes of Health, Bethesda, Maryland; http://imagej.nih.gov/ij/). The intensities of COX4 and COX1 bands were normalized to porin expression (Mitosciences) and expressed as ratios. Porin is a mitochondrial protein located in the outer membrane and used as a loading control. There were no significant differences in porin expression between NMX and HPX samples.

RNA Isolation, Complementary DNA Generation, and Real-Time Reverse Transcriptase–Polymerase Chain Reaction

Total RNA was isolated from left ventricles of NMX and HPX offspring hearts using Rneasy Fibrous Tissue total RNA isolation mini kit from Qiagen (Valencia, California). RNA samples were converted to complementary DNA (cDNA) using ABI High-Capacity cDNA Reverse Transcription kit. Briefly, 10 μL of 2× RT master mix are added to 10 μL of RNA (100 ng total). The reaction is then incubated at 37°C for 2 hours followed by a 5-minute incubation at 85°C to terminate the reaction. Quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR) reactions were conducted using Fermentas Maxima SYBR Green qPCR (Thermoscientific/Fermentas, Inc, Glen Burnie, Maryland). The primer sequences were obtained from Genebank and targeted COX4.1: (5′-TACACGTAGCGCTTCTCCCAGAT-3′; 5′-AGATGAACAGGGTCAGCAACCAGT-3′), COX4.2 (5′-GCCACCAAATCAGCAAAGCCGTTA-3′, 5′-TTCCGTGAGACCTTCGCAGAGATGAA-3′), COX5b (5′-CACCAGTTTGTAATGGGTTCCGCA-3′, 5′-AGGACAACAGCACTGTCATCTGGT-3′), PGC-1α (5′-AGTTCTGTCCGTGTTGTGTCAGGT-3′, 5′-ATCAGAAAG GGCCAAACAGAGGGA-3′), and NRF-1 (5′-GGCCGTTTC CGTTTCTTTCC TGTT-3′, 5′-TGGCCACTTACACTGAGCA-TAGCA-3′). The 40-cycle amplification protocol of cDNA samples was initiated by a 10-minute incubation at 95°C followed by 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Data were obtained from cycling time (CT) values (cycle number at which PCR product crosses threshold) quantified by the delta–delta CT (2-DDCt) method.[33] The mRNA levels for mitochondrial subunits (COX4.1, 4.2, 5b) and PGC-1α and NRF-1 were normalized to total RNA.

Statistics

Data are expressed as mean ± standard error of the mean. Student *t* test was used to analyze statistical significance between NMX and HPX groups. A *P* value of less than .05 was considered statistically significant.

Results

Animal Parameters

There were no differences in body weights (692.9 ± 30.4 vs 662.7 ± 13.3 g, NMX vs HPX, respectively), heart weights (1.95 ± 0.1 vs 1.84 ± 0.1 g), or heart weight–body weight ratios (0.0028 ± 0.001 vs 0.0028 ± 0.0001) between groups at 90 days of age despite previously reported fetal growth restriction of HPX guinea pig fetuses.[5-7,34] Offspring guinea pigs exposed to prenatal HPX (n = 9) had significantly ($P < .05$) greater systolic (59.2 ± 4.0 vs 72.3 ± 2.3 mm Hg, NMX vs HPX, respectively), diastolic (41.9 ± 3.4 vs 51.1 ± 1.8 mm Hg), and mean arterial blood pressures (47.1 ± 3.6 vs 58.2 ± 1.9 mm Hg) than that of NMX controls (n = 6). The arterial blood pressures of NMX are representative of values measured in anesthetized adult guinea pigs.[35,36]

Effects of Intrauterine HPX on Offspring Cytochrome Oxidase Activity

The CCO activity (oxidized cytochrome c/min/mg protein) is measured as an index of complex IV function. Mitochondrial CCO activity of offspring hearts of animals exposed to prenatal HPX and NMX is shown in Figure 1. Prenatal HPX decreased

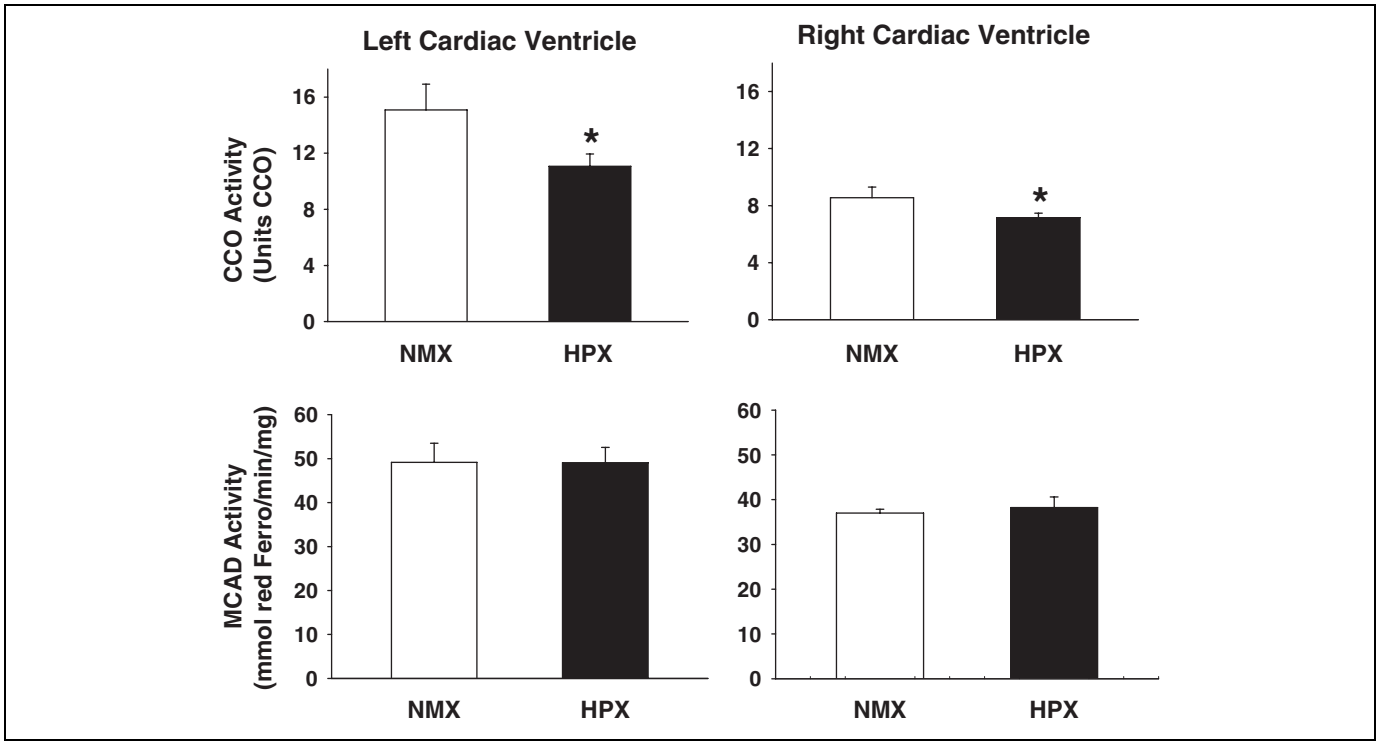

Figure 1. Cytochrome oxidase (CCO) and medium-chain acyl-coenzyme A dehydrogenase (MCAD) activity of left and right cardiac ventricles of offspring guinea pigs exposed to prenatal normoxia (NMX; n = 6) or hypoxia (HPX; n = 9). The CCO activity is expressed as units CCO (1 U = 1μmol of oxidized cytochrome c/min/mg protein) and MCAD activity as mmol of reduced ferrocenium/min/mg protein. Data are mean ± standard error of the mean (SEM).
* indicates $P < .05$ versus NMX controls.

the CCO activity in both the left (26.1%, $P < .05$) and the right ventricles (16.3%; $P < .05$) compared to NMX controls.

Effects of Intrauterine HPX on Offspring Medium-Chain Acyl-Coenzyme A Activity

Medium-chain acyl-coenzyme A dehydrogenase activity (reduced ferrocenium/min/mg protein) is measured as an index of fatty acid β-oxidation capacity. The MCAD activity levels of offspring heart ventricles are shown in Figure 1. The MCAD activity levels in either left or right ventricles were similar between NMX and HPX offspring hearts.

Effects of Chronic Intrauterine HPX on Expression of CCO Subunits 4 and 1

A nuclear-encoded subunit of CCO, COX4, has been demonstrated to play an important role in regulating the stability of the mitochondria-encoded catalytic CCO subunit 1 (COX1).[37] Figure 2 illustrates representative Western immunoblots of protein expression of COX4 and COX1 from cardiac left ventricles. Figure 2 presents the ratio of COX4 and COX1 protein expression (normalized to porin). Chronic intrauterine HPX reduces subunit expression of both COX4 and COX1 in cardiac left ventricles (by 57% and 51%, respectively; $P < .05$) compared to NMX controls.

Effects of Chronic Intrauterine HPX on Expression of CCO Subunit mRNA

Cytochrome C oxidase subunits 4.1, 4.2 (COX4.1 and COX4.2), and 5b are nuclear-encoded subunits of CCO, which regulate the stability and dimerization of complex IV.[37-39] In Figure 3, COX4.1 and 5b (by 22% and 21%, respectively; $P < .05$) but not COX 4.2 mRNA levels of cardiac left ventricles were decreased in HPX compared to NXM offspring hearts.

Effects of Chronic Fetal HPX on Transcriptional Coactivator Gene Expression

Both PGC-1α and NRF-1 are master regulators of mitochondrial gene expression and biogenesis.[38,39] Figure 4 illustrates mRNA expression levels in cardiac left ventricles of offspring hearts from animals exposed to intrauterine HPX. The PGC-1α mRNA levels were reduced ($P < 0.05$) by 14%, and the NRF-1 levels increased ($P < 0.05$) by 10% in offspring of HPX cardiac left ventricles.

Discussion

This study demonstrates that exposure to prenatal HPX reduces mitochondrial CCO activity in both the right and the left ventricles of 90-day-old male offspring. This was accompanied

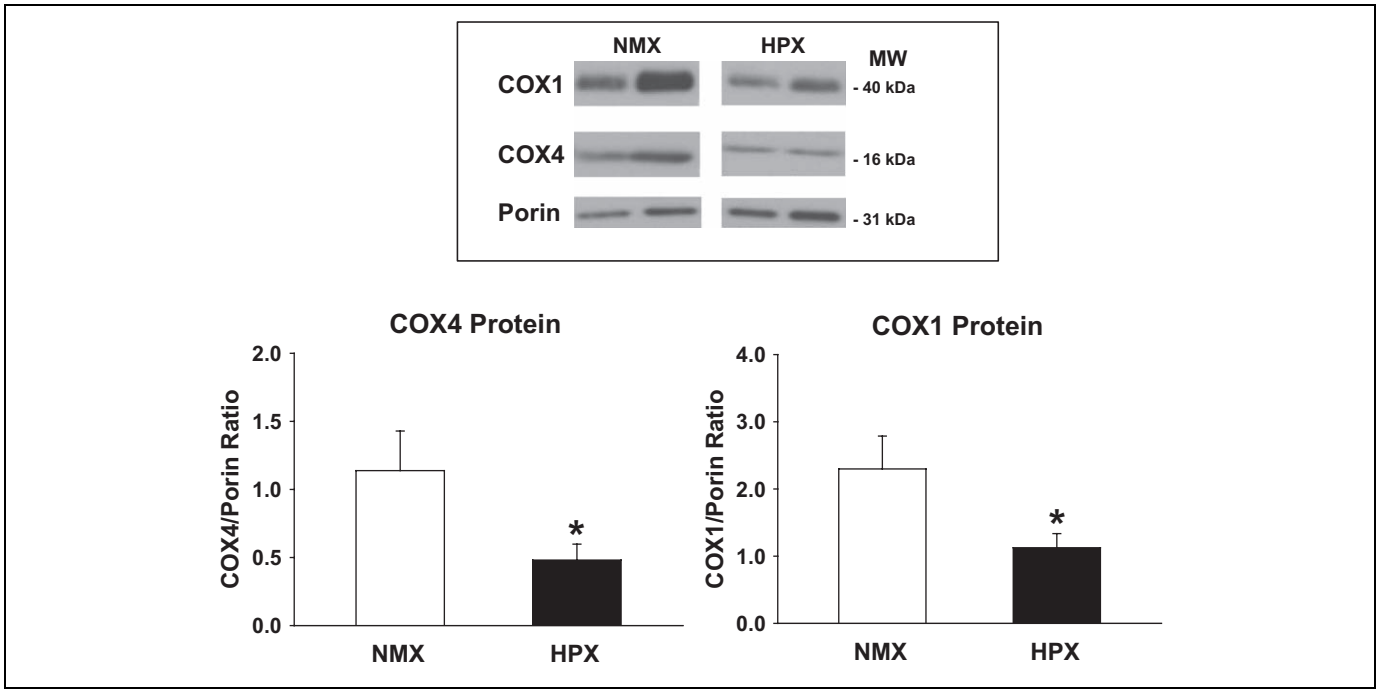

Figure 2. Protein levels of cytochrome oxidase subunits 4 and 1 (COX4 and COX1, respectively) of left cardiac ventricles of 90-day-old offspring guinea pigs exposed to prenatal normoxia (NMX; n = 4) or hypoxia (HPX; n = 5). COX4 and COX1 protein expression is measured by Western blot analysis as a ratio of COX4 or COX1/porin. Data are mean ± standard error of the mean (SEM).
* indicates $P < .05$ versus NMX controls.

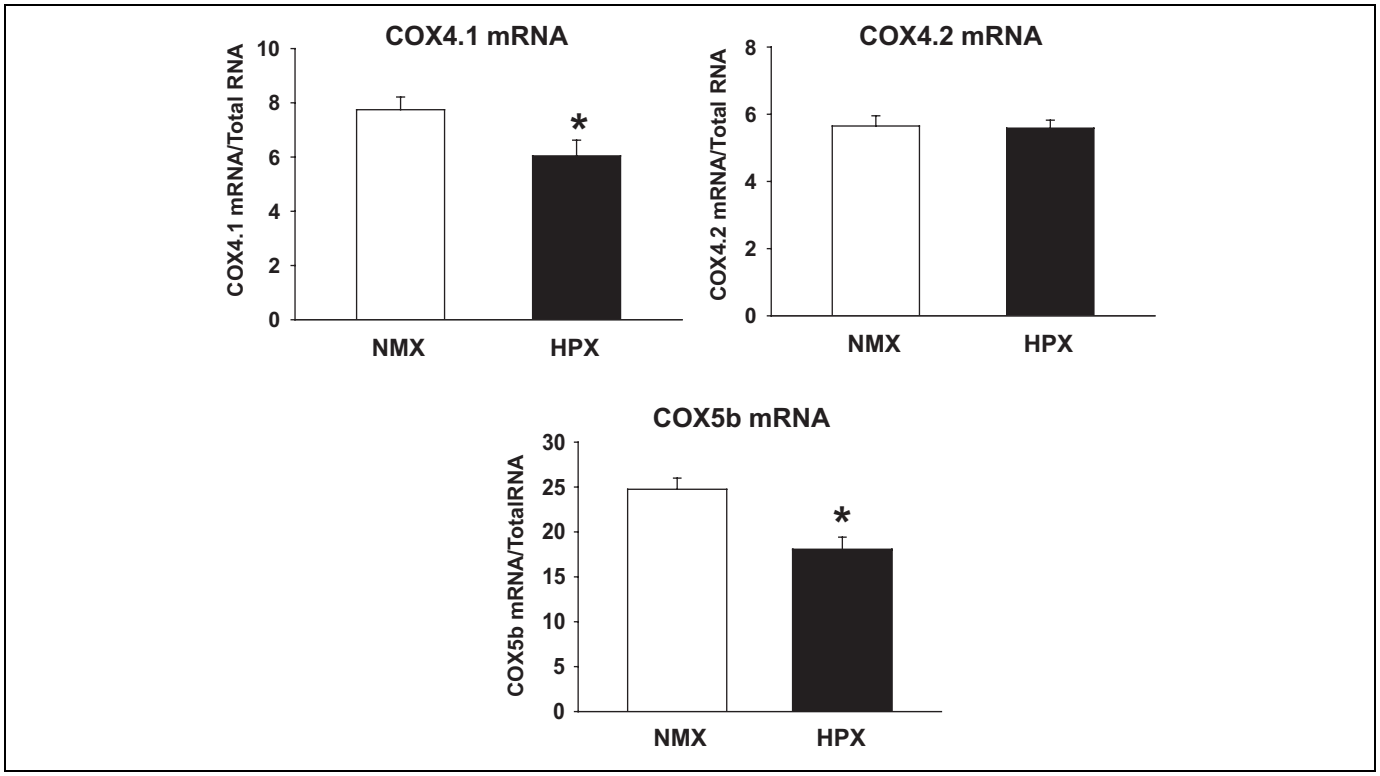

Figure 3. Messenger RNA (mRNA) expression of cytochrome oxidase subunits 4.1, 4.2, and 5b (COX4.1, COX4.2, and COX5b, respectively) in left cardiac ventricles of 90-day-old offspring guinea pigs exposed to prenatal normoxia (NMX) or hypoxia (HPX). COX4.1, 4.2, and COX5b mRNA expression levels are normalized to total RNA. Data are mean ± standard error of the mean (SEM).
* indicates $P < .05$ versus NMX controls. COX4.1 and 4.2 NMX n = 6, HPX n = 9, and COX5b NMX and HPX n = 5.

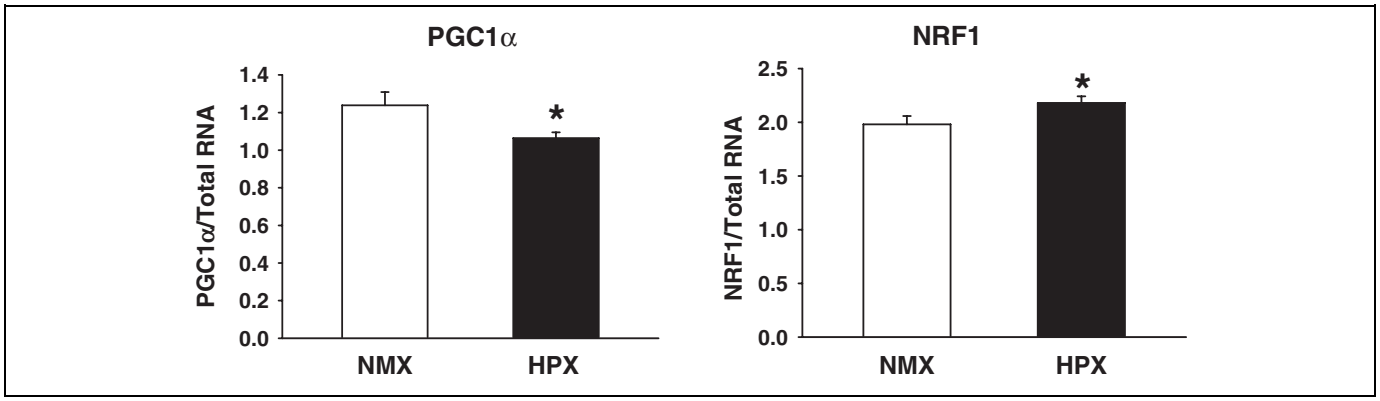

Figure 4. Peroxisome proliferator–activated receptor gamma coactivator 1-alpha (PGC-1α) and nuclear respiratory factor 1 (NRF-1) messenger RNA (mRNA) expression of left cardiac ventricles of 90-day-old offspring guinea pigs exposed to prenatal normoxia (NMX; n = 6) or hypoxia (HPX; n = 9). The expression levels of PGC-1α and NRF-1 transcript are normalized to total RNA and presented as a ratio of PGC-1α or NRF-1 levels/total RNA. Data are mean ± standard error of the mean (SEM).
* indicates $P < .05$ versus NMX controls.

by a concomitant decrease in the cardiac expression of nuclear- and mitochondrial-encoded CCO subunits, COX4 and COX1 proteins, respectively, of age-matched, prenatally exposed, HPX offspring compared to NMX controls. Prenatal HPX caused an isoform-specific reduction in COX5b and COX4.1 but not in COX4.2 mRNA expression and differentially altered mRNA levels of PGC-1α and NRF-1 in offspring hearts, with PGC-1α levels decreased and NRF-1 levels increased compared to controls. Taken together, these results indicate that intrauterine HPX downregulates both expression of mitochondrial CCO subunits and activity of left ventricles of 90-day-old offspring, consistent with decreased transcription of its target genes.

Cytochrome c oxidase is the terminal complex of the electron transport chain that facilitates the oxidation of cytochrome C by O_2. The mammalian CCO is made up of 13 subunits, 3 of which are mitochondrial encoded and the remaining 10 are encoded by the nuclear genome.[37] The CCO activity is regulated by transcription of both regulatory and catalytic subunits and is tightly coupled to the cell's energetic demand and metabolic output.[40-42] Both COX4, a regulatory subunit, and COX1, a catalytic subunit, are key proteins important in the regulation of CCO activity. The COX4 has been identified as an ATP-binding subunit,[43,44] and COX5b provides a binding site for protein kinase A.[45] The complex regulation of CCO activity arises in part from the product of the bigenomic contribution of nuclear and mitochondrial DNA, which encodes the protein subunits of CCO[41] and their assembly within the inner mitochondrial membrane.[40] The structure and activity of CCO is regulated by a variety of mechanisms including subunit assembly as well as allosteric modulation by small molecules such as peroxynitrite and by phosphorylation of specific CCO subunits.[37] We proposed that exposure to prenatal HPX would reduce mitochondrial protein expression and function in programmed offspring hearts. The decrease in expression of both COX4 and COX1 proteins measured in the present study is consistent with a decrease in CCO activity of offspring hearts exposed to prenatal HPX. In contrast, prenatal HPX did not alter MCAD activity, suggesting a selective alteration in mitochondrial function. In another study, a copper-deficient diet, fed to pregnant rats, decreased COX1 protein levels and CCO activity in heart ventricles of 21-day-old rat offspring,[28] linking mitochondrial protein expression and CCO activity as well as an inhibitory effect of intrauterine stress on cardiac mitochondria. Further, 2 infants who died of hypertrophic cardiomyopathy were identified with a homozygous mutation in *C2orf64*, an ortholog gene of yeast *PET191*, important in complex IV assembly.[46] Each exhibited impaired complex IV activity associated with decreased levels of fibroblast COX2, COX4, and COX5a subunits. Thus, the reduced expression of specific subunits of cardiac mitochondrial proteins of the electron transport chain may reduce enzyme activity important in mitochondrial respiration.

The molecular mechanisms associated with downregulation of mitochondrial protein expression in the offspring remain unclear. The PGC-1α is a major regulator of mitochondrial biogenesis and oxidative metabolism, inducing the transcription of mitochondrial proteins involved in oxidative phosphorylation and fatty acid oxidation.[38,41,42] It is abundantly expressed in heart tissue[47] and interacts with NRF-1 and NRF-2 to regulate heart energy metabolism.[42] The NRF-1 is a key regulator of nuclear-encoded subunits of CCO as well as all of the 5 respiratory complexes.[38,41] The NRF-2 plays a role in the expression of COX 4[48,49] and COX 5b[50,51] subunits along with other nuclear-encoded COX subunits.[52] The PGC-1α binds to NRF-1 and NRF-2, which is required for transactivation of NRF target genes.[53,54] It is a key regulator between environmental changes in the cell and their effects on gene expression.[39] Thus, PGC-1α serves an integrative function in binding to NRF-1 and NRF-2 and facilitating the activation of gene expression of mitochondrial protein subunits. The PGC-1α expression is regulated by HPX,[55] oxidative stress,[55-57] and hormone receptors,[41,58-60] and the loss of function of PGC-1α is associated with reduced mitochondrial ATP synthesis and fatty acid oxidation.[61] The downregulation of PGC-1α despite a small increase in NRF-1/2 may be sufficient

to contribute to the reduced expression of the downstream targets of NRF-1/2 if there is altered binding of the PGC-1α/NRF complex to the cytochrome subunit promoters. Thus, prenatal HPX exposure resulting in altered expression of NRFs and their coactivators may induce a dysregulation of mitochondrial protein expression that is sustained in the offspring.

There are several mechanisms by which mitochondrial protein expression may be downregulated by prenatal HPX in the offspring. These include epigenetic mechanisms of DNA methylation[62] and histone deacetylation as well as posttranslational modification. DNA methylation has been reported to be increased in response to both prenatal HPX in heart ventricles[63] and oxidative stress of cancer cells,[64] carotid body,[65,66] and the heart.[67] We have previously shown that prenatal HPX increases HIF-1α protein levels in HPX-exposed fetal hearts,[6] indicating local tissue HPX. Further, we have reported that fetal HPX increases fetal cardiac malondialdehyde levels and reduces mitochondrial CCO activity of fetal guinea pig hearts, both of which are reversed by maternal administration of the antioxidant, N-acetylcysteine, suggestive of oxidative stress.[27] Although there are no studies linking prenatal HPX and DNA methylation with decreased mitochondrial protein expression, conditions such as aging are associated with increased DNA methylation, decreased PGC-1α and COX7A1 mRNA, a cytochrome c subunit.[68] Alternatively, posttranslational modification can occur by direct inhibition of CCO activity by nitric oxide,[69,70] peroxynitrite,[71] malondialdehyde,[72] and acetylation of lysine residues.[73] Thus, the elevated nitric oxide, peroxynitrite, and malondialdehyde levels measured in HPX fetal guinea pig hearts in our previous studies[6,7] may be the initiating factors in generating a sustained decrease in CCO activity in the offspring. Previous studies have shown that both peroxynitrite and malondialdehyde can form adducts with mitochondrial complex activity in I, II, and V[71] and CCO,[72] respectively, to inhibit mitochondrial enzyme activity. Therefore, several signaling mechanisms initiated by intrauterine HPX may contribute to the altered mitochondrial protein activity measured in offspring hearts.

Finally, arterial blood pressure was increased in offspring exposed to prenatal HPX—a finding measured in some[74] but not all studies.[75,76] This indicates that prenatal HPX induces a cardiovascular risk of pressure overload in the offspring, in addition to a secondary risk of downregulation of mitochondrial protein expression in offspring hearts. Other studies have identified a downregulation of PGC-1α mRNA levels and mitochondrial energy metabolism following transverse aortic constriction and ventricular pressure overload in adult mouse hearts,[77,78] suggesting that increased blood pressure may have deleterious effects on hearts whose respiratory activity is compromised.

In summary, this study demonstrates that exposure to prenatal HPX alters mitochondrial protein expression and reduces CCO activity in offspring heart ventricles. This was attributed to downregulation of PGC-1α, a master regulator of mitochondrial protein expression, concomitant with a decrease in mRNA and protein levels of COX4 and COX1, key subunits of the CCO protein complex. These data suggest that offspring exposed to prenatal HPX may be at risk of mitochondrial dysfunction if intrauterine HPX induces permanent changes in cardiac mitochondrial protein expression and decreases electron transport chain function. Further study is needed to study the impact of altered mitochondrial protein expression on mitochondrial respiration and cardiac function with cardiovascular programming in the offspring.

Declaration of Conflicting Interests

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