Altered cardiolipin metabolism is associated with cardiac mitochondrial dysfunction in pulmonary vascular remodeled perinatal rat pups

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Abstract

Pulmonary vascular remodeling (PVR) in utero results in the development of heart failure. The alterations that occur in cardiac lipid and mitochondrial bioenergetics during the development of in utero PVR was unknown. In this study, PVR was induced in pups in utero by exposure of pregnant dams to indomethacin and hypoxia and cardiac lipids, echocardiographic function and cardiomyocyte mitochondrial function were subsequently examined. Perinatal rat pups with PVR exhibited elevated left and right cardiac ventricular internal dimensions and reduced ejection fraction and fractional shortening compared to controls. Cardiac myocytes from these pups exhibited increased glycolytic capacity and glycolytic reserve compared to controls. However, respiration with glucose as substrate was unaltered. Fatty acid oxidation and ATP-insensitive respiration were increased in isolated cardiac myocytes from these pups compared to controls. Although abundance of mitochondrial respiratory chain complexes was unaltered, increased trilinoleoyl-lysocardiolipin levels in these pups was observed. A compensatory increase in both cardiolipin and phosphatidylethanolamine content were observed due to increased synthesis of these phospholipids. These data indicate that alterations in cardiac cardiolipin and phospholipid metabolism in PVR rat pups is associated with the mitochondrial bioenergetic and cardiac functional defects observed in their hearts.

Introduction

Pulmonary hypertension of the newborn is a failure of normal pulmonary vascular relaxation after birth, with incidence of up to 6 per 1000 live births and 10–30% mortality [1–3]. It is a significant cause of heart failure (HF) in healthy newborn infants. In healthy term infants it is
caused by perinatal hypoxia, inflammation or direct lung injury. These infants develop the HF rapidly within a week and HF becomes the main limiting factor for their survival. A number of studies in human and animal models have demonstrated alterations in mitochondrial bioenergetics during the development of HF [4]. Several of these studies have implicated alterations in the mitochondrial phospholipid cardiolipin (CL) as a contributor to the mitochondrial dysfunction. CL, the signature phospholipid of mitochondria, is essential for mitochondrial morphology, bioenergetics, dynamics, and signaling pathways [5–11]. However, limited information exists on the changes in cardiac function and phospholipid composition that occur during in utero pulmonary vascular remodeling (PVR). Previously we demonstrated that newborn piglets exposed to a hypoxic environment for 3 days developed alterations in CL and reduced mitochondrial respiratory chain dysfunction during the development of a right ventricular hypertrophy [12].

In this study, we utilized a unique gestational rat model of PVR to examine how cardiac phospholipid metabolism, cardiac morphology and mitochondrial function are impacted by PVR. We show for the first time that CL and phosphatidylethanolamine (PE) levels are elevated in the hearts of PVR rat pups and that this is accompanied by an accumulation of lyso-cardiolipin (LysoCL) and mitochondrial bioenergetic and cardiac dysfunction.

### Materials and methods

#### Animals

This study was performed with approval of the University of Manitoba Animal Policy and Welfare Committee which adheres to the principles for biomedical research involving animals developed by the Canadian Council on Animal Care and the Council for International Organizations of Medical Sciences. This study is reported in accordance with ARRIVE guidelines. All animals were maintained in an environmentally controlled facility (22˚C, 37% humidity, 12 h light/dark cycle) with free access to food and water.

PVR was induced in perinatal rats by treating pregnant dams during 19–21 days of gestation with hypoxia and indomethacin (single ip dose 0.5 mg/Kg in sterile PBS pH 7.4) as described [13, 14]. Timed pregnant rats at 18 days gestation were utilized. One set of pregnant rats were housed normally (Control, single ip dose sterile PBS) as control animals, a second set of pregnant rats were indomethacin-treated as above (Indo), while a third set of pregnant rats were indomethacin-treated and then housed in a hypoxic environment (12% oxygen) (PVR). This was accomplished by housing rats in a plexiglass chamber and the chamber was maintained at 12% oxygen using a nitrogen washout system. Medical grade compressed oxygen (12% oxygen balance Nitrogen, Welder’s Supply) was used in combination with medical grade nitrogen to adjust chamber oxygen levels to 12% using a gas analyzer (Radiometer ABL 700 series). Compressed gas flowed continuously into the chamber at a rate of 1 L/min for 3 days. Chamber oxygen levels, flow rates and temperatures were monitored every 24 h at a minimum. On the fourth day all animals were administered isoflurane using a Bell jar/nose cone. Anesthesia was administered until the pedal withdrawal reflex was abolished at which time cervical dislocation was performed and then this was followed by rapid caesarean section to remove the pups. Prior to sacrifice fetal echocardiographic parameters were determined as described below. After removal by caesarean section the pups were then weighed, measured for length and the heart, liver and lung removed and weighed. Histology of pulmonary arteries was performed as previously described [12]. In some experiments, the heart was freeze dried and weighed. In other experiments, freshly isolated hearts were used for preparation of mitochondrial fractions or isolation of cardiac myocytes as outlined below.
**In vivo echocardiography**

Transthoracic echocardiography was performed on dams mildly anesthetized with 1–1.5% isoflurane, 1L/min oxygen as previously described [15]. Each rat was placed on a heated ECG platform to maintain body temperature and obtain fetal heart measurements. A Vevo 2100 High resolution imaging system equipped with a 30-MHz transducer (Visual Sonics, Toronto) was used to visualize the fetal hearts. Measurements obtained included interventricular septum diastolic (IVSd), interventricular septum systolic (IVSs), left ventricle internal dimension diastolic (LVIDd), left ventricle internal dimension systolic (LVIDs), left ventricular wall diastolic (LVWd), intraventricular septum end systole (LVSs), ejection fraction (EF), fractional shortening (FS), right ventricular wall diastolic (RVWd), right ventricular wall systolic (RVWs), right ventricle internal dimension diastolic (RVIDd) and right ventricle internal dimension systolic (RVIDs).

**Cardiac myocyte preparation and radiolabeling experiments**

Cardiac myocytes were prepared from the hearts of control and PVR pups as described [16]. The protocol allows for rat cardiomyocytes to be cultured for up to 72 h at 37°C in 5% CO₂ without significant change in phenotype [17]. Enough hearts were collected to yield approximately 15-20 million cardiac myocytes sufficient to plate 15–20 x 35mm dishes (1 million/dish, Corning Primaria™). Isolated cardiac myocytes were incubated with 0.1 mM [1,3-³H]glycerol (2 μCi/dish, Perkin Elmer) or 0.1 μM [1-¹⁴C]linoleic acid (2 μCi/dish, Perkin Elmer) bound to albumin (1:1 molar ratio) or 0.1 μM [1-¹⁴C]oleic acid (2 μCi/dish Perkin Elmer) bound to albumin (1:1 molar ratio) for up to 360 min (6 h) and radioactivity incorporated into phospholipids determined as previously described [18].

**Respiratory function analysis**

The oxygen consumption rate (OCR) was measured from isolated cardiac myocytes of control and PVR pups (1 x 10⁵/well, coated with fibronectin) using a Seahorse XF24 Bioscience instrument (18). XF assay media contained either 1 mmol/L pyruvate and 25 mmol/L glucose for glucose metabolism or 1 mmol/L pyruvate, 2.5 mmol/L glucose, 0.5 μmol/L carnitine, and 0.175 mmol/L palmitate-BSA for fatty acid (FA) metabolism. Basal oxygen consumption was considered to be the basal respiration sensitive to inhibition by 1 μmol/L antimycin A plus 1 μmol/L rotenone. ATP-sensitive oxygen consumption was inhibited by 1 μmol/L oligomycin, and ATP-insensitive respiration (heat) was the remaining proportion of basal oxygen consumption. Maximal oxygen consumption was achieved with 1 μmol/L carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone. Fatty acid-dependent respiration was measured as the difference in oxygen consumption measured in the presence of 40 μmol/L etomoxir and vehicle (water). The XF glycolysis stress test kit (Aligent) was used for glycolysis analysis. Briefly, cells are cultured in the absence of glucose followed by the sequential addition of glucose (10mM), oligomycin (3μM) and 2-DG (1M). Where glycolysis is measured following glucose addition (glucose—2-DG), glycolytic capacity measured following oligomycin addition (oligomycin—2-DG), and glycolytic capacity the difference between them (glycolytic capacity—glucose).

**Western blot analysis of mitochondrial respiratory subunits**

Cardiac mitochondria were isolated from control or PVR hearts using the MITOISO1 Mitochondria Isolation Kit (Sigma) as previously described [15]. Mitochondrial protein (7.5 μg) from was separated on the Bio-Rad mini gel electrophoresis system by SDS-PAGE (12%...
acrylamide) as previously described [15]. Western blot antibody cocktail (Abcam) contained (55 kDa, anti-ATP synthase subunit ATP5a, Complex V; 47 kDa, anti-complex III subunit core 2 UQCRC2; 35 kDa, anti-complex IV subunit MTCO1; 30 kDa, complex II subunit SDHB; and 20 kDa, complex I subunit NDUFB8). Adult rat mitochondrial protein was added for comparative control and α-tubulin (Cell Signaling) was used as the loading control. Proteins were visualized by chemiluminescence using the ECL Western blotting detection system (Amersham).

Lipid analysis
The level of the major phospholipids from cardiac tissue homogenates including phosphatidylcholine (PC), PE and CL were determined by HPLC separation followed by lipid phosphorus assay [19, 20]. Molecular species of CLs and lysoCLs were quantitated from tissue homogenates by HPLC coupled to electrospray ionization mass spectrometry [21]. Cardiac cholesterol, cholesterol ester and triacylglycerol were determined by HPLC as previously described [15].

Statistical analysis
The randomization of the groups for each of the experiments is outlined in S1 Fig. For body and tissue weights, statistics were performed on n = 64–68 pups per group (from 4 different dams). The histology was performed on the first litter of this series from the control and PVR group (6 and 11 pups analyzed, respectively). The echocardiography was performed on an entirely different set of dams. For the control group 6 pregnant dams were used to collect left ventricle measurements from a total of 31 fetuses in utero. The PVR groups used 7 pregnant dams to collect left ventricle measurements from 37 fetuses in utero. The right ventricle measurements were technically difficult and as a result fewer animals were used; 21 fetuses (4 dams) from the control group and 18 fetuses (5 dams) from the PVR group. Data are expressed as means ± standard error of the mean (SEM). Comparisons between control and PVR offspring were determined using 1-way analysis of variance using Tukey post-hoc analysis. For each measurement, the offspring were derived from multiple litters. A probability p value of <0.05 was considered significant.

Results
PVR was induced in perinatal rats by treating pregnant dams during 19–21 days of gestation with hypoxia and indomethacin [13, 14]. Lung histology analysis of newborn rats revealed classic PVR in pups from the hypoxic-indomethacin treated group compared to control including increased medial thickness in the larger pulmonary arteries with no changes in their external diameter (Fig 1A–1D). Thus, we established this model of PVR in fetal rats [13, 14]. Reduction in body weight and length of newborn PVR pups were accompanied by reductions in both lung and liver weight compared to control (Fig 2A–2D). Dried heart weight/body weight ratio was elevated in PVR pups compared to control (Fig 2E) and this was accompanied alterations in cardiac structural and functional parameters (Fig 3A–3C). Specifically, in the left ventricle elevations in IVSd, IVSs, LVWd and LVSs were accompanied by a reduction in EF and FS. In addition, right ventricle dimensions including RVWd and RVWs were elevated in PVR pups compared to control. Thus, the PVR rat hearts exhibited cardiac hypertrophy and mechanical dysfunction.

We examined if substrate utilization was altered in isolated cardiac myocytes from rats in which PVR was induced during gestation. Oxygen consumption rate (OCR) was unaltered in PVR pups compared to control when glucose was used as substrate (S2 Fig). Interestingly, glycolytic capacity and glycolytic reserve were increased in PVR pups compared to control.
In addition, when palmitate was used as substrate, an increase in basal OCR was observed which was blocked by incubation with the carnitine palmitoyltransferase-1 inhibitor etomoxir (Fig 4B). The increase in fatty acid (FA) oxidation was accompanied an increase in ATP-sensitive oxygen consumption (Fig 4C) and ATP-insensitive respiration (Fig 4D) in PVR pups compared to control. Maximum OCR was unaltered in cardiac myocytes between control and PVR pups (Fig 4E). Thus, the increase in ATP-insensitive respiration indicated a dysfunction in efficiency of mitochondrial FA oxidation in cardiac myocytes from PVR rat pups.

We next examined the pool sizes of the three major phospholipids in hearts from control and PVR pups. Phospholipid analysis revealed that there was no alteration in the levels of phosphatidylcholine (PC) but striking increases in both cardiolipin (CL) and phosphatidylethanolamine (PE) in hearts of PVR pups compared to control (Fig 5A–5C). The observed reduction in cardiac PC/PE ratio was consistent with that seen in pressure-induced heart failure [22]. In addition, the levels of cardiac cholesterol, cholesterol ester and triacylglycerol were unaltered (S3 Fig). Since CL, and specifically tetralinoleoylcardiolipin (L4-CL), are required for

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**Fig 1. Histology of PVR arteries.** Masson’s trichrome staining of control (A) and PVR (B) pulmonary arteries. Representative sections are depicted. C. Medial thickness, and D. External diameter of control and PVR pulmonary arteries. The black bar insert represents 50 μM. Data represent the mean ± SEM, *p<0.05. N values are indicated in S1 Fig.

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optimal cardiac mitochondrial bioenergetic function \[23\], we examined the molecular species composition of CL. Elevations in 1442, 1448 and 1472 species of CL appeared to be responsible for the observed increase in CL in PVR pups (Fig 5D). The highest being linoleate containing L4CL (1448). In isolated cardiac myocytes from PVR pups the increase in L4CL was due to an increase in [1,14C]linoleate incorporation into CL indicating increased synthesis from linoleate (Fig 6A). In contrast, de novo synthesis of CL from [1,3-3H]glycerol was unaltered in cardiac myocytes from PVR rat pups (Fig 6B).

Fig 2. Body weight, length and organ weight of PVR rats. Whole body weight (A), length (B), Liver weight (C) and lung weight (D) of control and PVR rat pups. E. Heart weight and dry heart weight/body weight ratio of control and PVR rats. Data represent the mean ± SEM, *p<0.05. N values are indicated in S1 Fig.

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Since the increase in L\textsubscript{4}CL did not explain the mitochondrial dysfunction, we examined the levels of cardiac mitochondrial respiratory complexes. No alteration in the abundance of mitochondrial complexes was observed in PVR pups compared to controls (S2 Fig). LysoCL accumulation is known to cause mitochondrial bioenergetic dysfunction [24]. We observed elevated trilinoleoyl-lysoCL (L\textsubscript{3}-lysoCL) species in hearts of PVR pups compared to controls (Fig 5E). Thus, an elevation in linoleate containing L\textsubscript{3}-lysoCL species could be linked to the observed cardiac mitochondrial bioenergetic dysfunction in PVR rat pups.

The reason for the elevation in PE was also examined. Incorporation of radioactivity into PE from [1,3-\textsuperscript{3}H]glycerol was unaltered in isolated cardiomyocytes from PVR rat pups indicating that de novo synthesis was unaltered (Fig 6B). To further confirm this, synthesis of PE from serine and ethanolamine were examined. There was no alteration in synthesis of PE from \textsuperscript{3}H]ethanolamine nor synthesis of PE or phosphatidylserine from \textsuperscript{3}H]serine in cardiac
myocytes from PVR rat pups (S4 Fig). In contrast, the increase in PE was due to an increase in \([1^{14}C]\)oleate incorporation into PE by 6 h of incubation indicating increased synthesis from oleate (Fig 6C).
Discussion

In this study, we examined how altered cardiac CL and phospholipid metabolism and mitochondrial dysfunction are associated with the perinatal cardiac pathophysiology of PVR in perinatal rat pups. We show that PVR in perinatal rat pups result in cardiac hypertrophy and ventricular dysfunction, cardiac myocyte mitochondrial dysfunction with altered FA substrate utilization and elevated CL, L\textsubscript{3}-LysoCL and PE which may contribute, in part, to the cardiac mitochondrial dysfunction.
In this study, we have characterized for the first time the cardiac lipid alterations that occur in pups of the in utero hypoxia and indomethacin-induced fetal PVR rat model [13, 14]. There are several reports of indomethacin induced pulmonary hypertension in human newborns mediated by premature constriction of the fetal ductus arteriosus [25–28]. In addition, indomethacin-mediated closure of the ductus arteriosus in fetal rats was shown to result in early-onset right ventricular hypertrophy [29]. Perinatal pulmonary hypertension in rats is known to permanently modify the pulmonary vasculature [30, 31]. Consistent with this we observed that pulmonary arteries from our pups exhibited increased medial thickness in the larger pulmonary arteries with no changes in their external diameter. Associated with the pulmonary arterial modification was a cardiac hypertrophy characterized by elevation in right ventricle dimensions including RVWd and RVWs. In addition, left ventricle elevations in IVSd, IVSs, LVWd and LVs, and reduction in EF and FS were observed. The elevation in right ventricular dimensions were consistent with that previously reported in this model of PVR [32].

Alteration in substrate utilization is a hallmark of cardiac dysfunction in HF [33]. Studies in animal models and in human pulmonary arterial hypertension suggest that there is increased...
glycolysis and a metabolic shift from oxidative mitochondrial metabolism to the less energy efficient glycolytic metabolism [34, 35]. Although basal OCR was unaltered in isolated cardiac myocytes of PVR pups when glucose was used as substrate, we observed increased glycolytic capacity and glycolytic reserve suggesting the potential for increase in glucose utilization for ATP synthesis. When palmitate was used as substrate an increase in FA oxidation was accompanied by an increase in ATP-insensitive respiration in isolated cardiac myocytes of PVR pups.

Pressure induced cardiac failure in rodents is known to result in increased PE levels and cardiac dysfunction [22]. In addition, right ventricular pressure overload in adult rats induced by 12 weeks pulmonary arterial banding resulted in elevations in PE [34]. We observed elevated cardiac PE levels, with no changes in PC, in hearts of perinatal PVR rat pups. The observed reduction in cardiac PC/PE ratio is consistent with that seen in pressure-induced heart failure [22]. The increase in PE was not due to increased de novo synthesis or decarboxylation from phosphatidylserine since PE synthesis from radiolabeled glycerol, ethanolamine or serine was unaltered. In contrast, pulse-labeling with [1–14]oleate revealed increased synthesis of PE from oleate was responsible for the accumulation of PE in isolated cardiac myocytes from PVR rat pups.

A key question is whether alteration in CL levels actually contribute to the development of pediatric HF [36]. A number of adult animal and human studies have indicated that reduced CL and L₄CL accompany the development of HF [4, 37, 38]. The reduction in cardiac CL in many early studies of adult HF in rodents can be attributed to the feeding of defined diets which may subject these animals to accelerated HF [39]. The animals used in our study were harvested by caesarean section and were not subjected to maternal feeding. It was recently demonstrated that the relative percentage of L₄CL was preserved in pediatric human congenital single ventricle heart disease samples relative to biventricular controls [40]. In addition, differences in CL content were not observed in induced pluripotent stem cell cardiac myocytes prepared from control and pediatric dilated cardiomyopathy with ataxia syndrome patients [41]. Another study observed that the CL profiles in pediatric HF were unique from those in adults and the authors of this study hypothesized that end-stage pediatric heart failure adaptive mechanisms to preserve L₄CL content may be intact to a greater degree than that seen in adult heart failure [42]. Our results support the above hypothesis as we observed increased CL and L₄CL levels in the hearts of caesarean section harvested perinatal PVR rat pups. Although de novo synthesis of CL from glycerol was unaltered in cardiac myocytes of perinatal PVR rat pups, an increase in [1–14]C]linoleate incorporation into CL was observed which would explain the accumulation of CL and L₄lysoCL. The elevation in L₄CL might contribute to the observed increase in FA oxidation in isolated cardiac myocytes from these animals. However, the apparent compensatory increase in FA oxidation in isolated cardiac myocytes from PVR rat pups was accompanied by an increased state 4 (oligomycin-inhibited) respiration indicative of an elevated proton leak which likely contributes to the mitochondrial dysfunction.

Accumulation of lysoCL is known to result in mitochondrial bioenergetic dysfunction by compromising the stability of the protein-dense mitochondrial inner membrane leading to a decrease in optimal respiration [24]. Although we observed no alteration in the abundance of individual mitochondrial complexes subunits, the significant accumulation of L₃lysoCL observed in the hearts of perinatal PVR rat pups might contribute to the mitochondrial bioenergetic dysfunction through a morphological disturbance of cristae membrane structure. This will form the basis of a future study examining the mitochondrial morphology of cardiac myocytes from PVR animals.

A limitation of the use of this hypoxia-induced model of PVR is whether the observed effects on metabolism may simply be due to the hypoxia itself. However, while hypoxia may
cause a global change in cardiac homeostasis, the perinatal rats used in our study exhibited the cumulative effects of pressure, and the additional increase in cardiac weight was due to the effect of increased afterload. In addition, it is possible that the metabolic effects we observed may be more prominent in the right ventricle than the left ventricle. However, metabolism is also abnormal in the left ventricle in pulmonary arterial hypertension [43].

In summary, our data show for the first time that a perturbed CL and PE metabolism is associated with and may contribute, in part, to the mitochondrial bioenergetic and cardiac functional defects observed in the heart of the hypoxia and indomethacin-induced gestational model of perinatal PVR in rats.

Supporting information

S1 Fig. Randomization of pups for experimental analysis.
(TIF)

S2 Fig. Mitochondrial respiration and mitochondrial complexes in PVR cardiac myocytes.
(TIF)

S3 Fig. Cholesterol ester, triacylglycerol, and free cholesterol in PVR rat hearts.
(TIF)

S4 Fig. Synthesis of PE from ethanolamine or serine.
(TIF)

S1 Data.
(XLSX)

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References

1. Therese P. Persistent pulmonary hypertension of the newborn. Paediatr Respir Rev. 2006; 7: Suppl 1: S175–S176. https://doi.org/10.1016/j.prrv.2006.04.211 PMID: 16798554
2. Vosatka RJ. Persistent pulmonary hypertension of the newborn. N Engl J Med. 2002; 346: 864. https://doi.org/10.1056/NEJM200203143461117 PMID: 11893805
3. Dakshinamurtti S. Pathophysiologic mechanisms of persistent pulmonary hypertension of the newborn. Pediatr Pulmonol. 2005; 39: 492–503. https://doi.org/10.1002/ppul.20201 PMID: 15789439
4. Dolinsky VW, Cole LK, Sparagna GC, Hatch GM. Cardiac mitochondrial energy metabolism in heart failure: Role of cardiolipin and sirtuins. Biochim Biophys Acta. 2016; 1861: 1544–1554. https://doi.org/10.1016/j.bbabio.2016.03.008 PMID: 26972373
5. Claypool SM, Koehler CM. The complexity of cardiolipin in health and disease. Trends Biochem Sci. 2012; 37: 32–41. https://doi.org/10.1016/j.tibs.2011.09.003 PMID: 22014644
6. Falabella M, Vernon HJ, Hanna MG, Claypool SM, Pitceathly RDS. Cardiolipin, Mitochondria, and Neurological Disease. Trends Endocrinol Metab. 2021; 32: 224–237. https://doi.org/10.1016/j.tem.2021.01.006 PMID: 33640250
7. Ikon N, Ryan RO. Barth Syndrome: Connecting Cardiolipin to Cardiomyopathy. Lipids 2017; 52: 99–108. https://doi.org/10.1007/s11745-016-4229-7 PMID: 28070695
8. Ren M, Phoon CK, Schlame M. Metabolism and function of mitochondrial cardiolipin. Prog Lipid Res. 2014; 55: 1–16. https://doi.org/10.1016/j.plipres.2014.04.001 PMID: 24769127
9. Shen Z, Ye C, Mc Cain K, Greenberg ML. The Role of Cardiolipin in Cardiovascular Health. Biomed Res Int. 2015; 891707. https://doi.org/10.1155/2015/891707 PMID: 26301254
10. Wasmus C, Dudek J. Metabolic Alterations Caused by Defective Cardiolipin Remodeling in Inherited Cardiomyopathies. Life (Basel). 2020; 10: 277. https://doi.org/10.3390/life10110277 PMID: 33187128
11. Zegallai HM, Hatch GM. Barth syndrome: cardiolipin, cellular pathophysiology, management, and novel therapeutic targets. Mol Cell Biochem. 2021; 76: 1605–1629.
12. Saini-Chohan HK, Dakshinamurtti S, Taylor WA, Shen GX, Murphy R, Sparagna GC. et al. Persistent pulmonary hypertension results in reduced tetralinoleoyl-cardiolipin and mitochondrial complex II + III during the development of right ventricular hypertrophy in the neonatal pig heart. Am J Physiol Heart Circ Physiol. 2011; 301: H1415–H1424. https://doi.org/10.1152/ajpheart.00247.2011 PMID: 21841017
13. Xu XF, Gu WZ, Wu XL, Li RY, Du LZ. Fetal pulmonary vascular remodeling in a rat model induced by hypoxia and indomethacin. J Matern Fetal Neonatal Med. 2011; 24: 172–182. https://doi.org/10.3109/14767058.2010.482608 PMID: 20459333
14. Xu XF, Ma XL, Shen Z, Wu XL, Cheng F, Du LZ. Epigenetic regulation of the endothelial nitric oxide synthase gene in persistent pulmonary hypertension of the newborn rat. Hypertens. 2010; 28: 2227–2235. https://doi.org/10.1097/HJH.0b013e32835e0f1 PMID: 20724942
15. Cole LK, Mejia EM, Sparagna GC, Vandel M, Xiang B, Han X. et al. Cardiolipin deficiency elevates susceptibility to a lipotoxic hypertrophic cardiomyopathy. J Mol Cell Cardiol. 2020; 144: 24–34. https://doi.org/10.1016/j.yjmcc.2020.05.001 PMID: 32418915
16. Kovacic S, Soltys CL, Barr AJ, Shiojima I, Walsh K, Dyck JR. Akt activity negatively regulates phosphorylation of AMP-activated protein kinase in the heart. J Biol Chem. 2003; 278: 39422–39427. https://doi.org/10.1074/jbc.M305371200 PMID: 12890675
17. Chan AY, Soltys CL, Young ME, Proud CG, Dyck JR. Activation of AMP-activated protein kinase inhibits protein synthesis associated with hypertrophy in the cardiac myocyte. J Biol Chem 2004; 279: 32771–32779. https://doi.org/10.1074/jbc.M403528200 PMID: 15159410
18. Hatch G, McClarty G. Regulation of cardiolipin biosynthesis in H9c2 cardiac myoblasts by cytidine 5’-triphosphate. J Biol Chem. 1996; 271: 25810–25816. https://doi.org/10.1074/jbc.271.42.25810 PMID: 8824210
19. Cole LK, Mejia EM, Vandel M, Sparagna GC, Claypool SM, Dyck-Chan L. et al. Impaired Cardiolipin Biosynthesis Prevents Hepatic Steatosis and Diet-Induced Obesity. Diabetes. 2016; 65: 3289–3300. https://doi.org/10.2337/db16-0114 PMID: 27495222
20. Rouser G, Siakotos AN, Fleischer S. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. Lipids. 1966; 1: 85–86. https://doi.org/10.1007/BF02668129 PMID: 17805690
21. Sparagna GC., Johnson CA., McCune SA., Moore RL. Murphy RC. Quantitation of cardiolipin molecular species in spontaneously hypertensive heart failure rats using electrospray ionization mass spectrometry. J Lipid Res. 2005; 46: 1196–204. https://doi.org/10.1194/jlr.M500031-JLR200 PMID: 15772420

22. Slatzki J, Foryst-Ludwig A, Benteke K, Blumrich A, Smeir E, Ban Z. et al. Adipose tissue ATGL modifies the cardiac lipidome in pressure-overload-induced left ventricular failure. PLoS Genet 2018; 14: 98–106. https://doi.org/10.1371/journal.pgen.1007171 PMID: 29320510

23. Mejia EM, Cole PK, Hatch GM. Cardiolipin metabolism and the role it plays in heart failure and mitochondrial supercomplex formation. Cardiovasc Hematol Disord Drug Targets. 2014; 14: 98–106. https://doi.org/10.2174/1871529x14666140505123753 PMID: 24801725

24. Duncan AL. Monolysocardiolipin (MLCL) interactions with mitochondrial membrane proteins. Biochem Soc Trans. 2020; 48: 993–1004. https://doi.org/10.1042/BST20190932 PMID: 32453413

25. Lozano González CH, Jiménez PG, Pezzotti MA, Favela EL. Persistent pulmonary hypertension in the newborn caused by prenatal use of prostaglandin inhibitors, (indomethacin). Ginecol Obstet Mex. 1980; 48: 103–110. PMID: 7450523

26. Moise KJ Jr, Huhta JC, Sharif DS, Ou CN, Kirshon B, Wasserstrum N. et al. Indomethacin in the treatment of premature labor. Effects on the fetal ductus arteriosus. N Engl J Med. 1988; 319: 327–331. https://doi.org/10.1056/NEJM198808113190602 PMID: 3393194

27. Rudolph AM. The effects of nonsteroidal antiinflammatory compounds on fetal circulation and pulmonary function. Obstet Gynecol. 1981; 58(S Suppl): 63S–67S. PMID: 7312230

28. Tarcan A, Gürakan B, Yıldırım S, Ozkiraz S, Bilezikçi B. Persistent pulmonary hypertension in a premature newborn after 16 hours of antenatal indomethacin exposure. J Perinat Med. 2004; 32: 98–99. https://doi.org/10.1515/JPM.2004.019 PMID: 15008397

29. Fabris VE, Pato MD, Belik J. Progressive lung and cardiac changes associated with pulmonary hypertension in the fetal rat. Pediatr Pulmonol. 2001; 31: 344–353. https://doi.org/10.1002/ppul.1057 PMID: 11340680

30. Harker LC, Kirkpatrick SE, Friedman WF, Bloor CM. Effects of indomethacin on fetal rat lungs: a possible cause of persistent fetal circulation (PFC). Pediatr Res. 1981; 15: 147–151. https://doi.org/10.1203/00006450-198102000-00013 PMID: 7196010

31. Herget J, Hampl V, Povýšilová V, Slavík Z. Long-term effects of prenatal indomethacin administration on the pulmonary circulation in rats. Eur Respir J. 1995; 8: 209–215. https://doi.org/10.1183/09031936.95.08020209 PMID: 7758553

32. Du Y, Fu J, Yao L, Qiao L, Liu N, Xing Y. et al. Altered expression of PPARγ and TRPC in neonatal rats with persistent pulmonary hypertension. Mol Med Rep. 2017; 16: 1117–1124. https://doi.org/10.3892/mmr.2017.6744 PMID: 28627661

33. Murashige D, Jang C, Neinast M, Edwards JJ, Cowan A, Hyman MC. et al. Comprehensive quantification of fuel use by the failing and nonfailing human heart. Science 2020; 370: 364–368. https://doi.org/10.1126/science.abc8861 PMID: 3306364

34. Koop AC, Bosser GPL, Ploegstra MJ, Hagdorn QAJ, Berger RMF, Sillijë HHW. et al. Metabolic Remodeling in the Pressure-Loaded Right Ventricle: Shifts in Glucose and Fatty Acid Metabolism- A Systematic Review and Meta-Analysis. J Am Heart Assoc. 2019; 8: e012086. https://doi.org/10.1161/JAHA.119.e012086 PMID: 31657265

35. Piao L, Marsboom G, Archer SL. Mitochondrial metabolic adaptation in right ventricular hypertrophy and failure. J Mol Med (Berl). 2010; 88: 1011–1020. https://doi.org/10.1007/s00109-010-0679-1 PMID: 20820751

36. Fillmore N, Lopaschuk GD. The link between pediatric heart failure and mitochondrial lipids. J Mol Cell Cardiol. 2014; 76: 71–72. https://doi.org/10.1016/j.yjmcc.2014.08.002 PMID: 25123339

37. Sparagna GC, Chicco AJ, Murphy RC, Bristow MR, Johnson CA, Rees ML. et al. Loss of cardiac tetra-unsaturated cardiolipin in human and experimental heart failure. J Lipid Res. 2007; 48: 1559–1570. https://doi.org/10.1194/jlr.M600551-JLR200 PMID: 17426348

38. Yoshizawa T, Sakurai T, Kawai K, Ichikawa-Shindo Y, Kawate H, Iesato Y. et al. Altered expression of PPAR-γ and TRPC in neonatal rats with persistent pulmonary hypertension. Mol Med Rep. 2017; 16: 1117–1124. https://doi.org/10.3892/mmr.2017.6744 PMID: 28627661

39. Rees ML. Gioscia-Ryan RA, McCune SA, Browder JC, Zachman DK. Chicco AJ. et al. The AIN-76A defined rodent diet accelerates the development of heart failure in SHHF rats: A cautionary note on its use in cardiac studies. J Animal Physiol Nutr. 2014; 98: 56–64. https://doi.org/10.1111/jjp.12031 PMID: 23298172
40. Garcia AM, McPhaul JC, Sparagna GC, Jeffrey DA, Jonscher R, Patel SS. et al. Alteration of cardiolipin biosynthesis and remodeling in single right ventricle congenital heart disease. Am J Physiol Heart Circ Physiol. 2020; 318: H787–H800. https://doi.org/10.1152/ajpheart.00494.2019 PMID: 32056460

41. Rohani L, Machiraju P, Sabouny R, Meng G, Liu S, Zhao T. et al. Reversible Mitochondrial Fragmentation in iPSC-Derived Cardiomyocytes From Children With DCMA, a Mitochondrial Cardiomyopathy. Can J Cardiol. 2020; 36: 554–563. https://doi.org/10.1016/j.cjca.2019.09.021 PMID: 32046906

42. Chatfield KC, Sparagna GC, Sucharov CC, Miyamoto SD, Grudis JE, Sobus RD. et al. Dysregulation of cardiolipin biosynthesis in pediatric heart failure. J Mol Cell Cardiol. 2014; 74: 251–259. https://doi.org/10.1016/j.yjmcc.2014.06.002 PMID: 24937604

43. Ryan JJ, Archer SL. Emerging concepts in the molecular basis of pulmonary arterial hypertension: part I: metabolic plasticity and mitochondrial dynamics in the pulmonary circulation and right ventricle in pulmonary arterial hypertension. Circulation. 2015; 131: 1691–702. https://doi.org/10.1161/CIRCULATIONAHA.114.006979 PMID: 25964279