Research Paper

Coenzyme Q supplementation in pulmonary arterial hypertension

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ABSTRACT

Mitochondrial dysfunction is a fundamental abnormality in the vascular endothelium and smooth muscle of patients with pulmonary arterial hypertension (PAH). Because coenzyme Q (CoQ) is essential for mitochondrial function and efficient oxygen utilization as the electron carrier in the inner mitochondrial membrane, we hypothesized that CoQ would improve mitochondrial function and benefit PAH patients. To test this, oxidized and reduced levels of CoQ, cardiac function by echocardiogram, mitochondrial functions of heme synthesis and cellular metabolism were evaluated in PAH patients ($N=8$) in comparison to healthy controls ($N=7$), at baseline and after 12 weeks oral CoQ supplementation. CoQ levels were similar among PAH and control individuals, and increased in all subjects with CoQ supplementation. PAH patients had higher CoQ levels than controls with supplementation, and a tendency to a higher reduced-to-oxidized CoQ ratio. Cardiac parameters improved with CoQ supplementation, although 6-minute walk distances and BNP levels did not significantly change. Consistent with improved mitochondrial synthetic function, hemoglobin increased and red cell distribution width (RDW) decreased in PAH patients with CoQ, while hemoglobin declined slightly and RDW did not change in healthy controls. In contrast, metabolic and redox parameters, including lactate, pyruvate and reduced or oxidized gluthathione, did not change in PAH patients with CoQ. In summary, CoQ improved hemoglobin and red cell maturation in PAH, but longer studies and/or higher doses with a randomized placebo-controlled controlled design are necessary to evaluate the clinical benefit of this simple nutritional supplement.

Introduction

Pulmonary arterial hypertension (PAH) is a fatal disease characterized by a proliferative panvasculopathy that leads to right-sided heart failure. Abnormalities of mitochondrial function are mechanistically associated with the pulmonary vascular and cardiac disease [1–4]. Mitochondria are the principal source of energy for all cells through oxidative phosphorylation, and the main site for heme synthesis. Iron is incorporated into the protoporphyrin ring by mitochondrial ferrochelatase to produce heme and ultimately hemoproteins, such as hemoglobin. Abnormalities of mitochondrial oxygen utilization, energy production, and heme synthesis are all well described in PAH patients and have been linked to the underlying pathophysiology contributing to the disease [2,3,5–7]. Consistent with abnormalities of heme synthesis and hemoglobin production, PAH patients have greater variation in the size distribution of red blood cells, i.e. an increase in the red cell distribution width (RDW). In fact, studies show that RDW is a prognostic marker of pulmonary artery pressures, heart failure and mortality in PAH patients [8,9], and is independent of the B-type natriuretic protein (BNP), a validated biomarker of

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myocardial stress [8]. In addition to synthetic and energetic functions, mitochondria are also a primary site for production of reactive oxygen species, as a byproduct of the electron transport reactions, which contribute to injury and inflammation of the pulmonary vasculature in PAH [10]. Taken together, the evidence suggests that interventions aimed at improvement of mitochondrial function might benefit PAH.

Coenzyme Q (CoQ) is one of the most widely used cofactors for treating mitochondrial-related diseases. CoQ has an important role in mitochondrial metabolism by functioning as the electron carrier in the inner mitochondrial membrane, transferring electrons from complexes I and II to complex III [11], but CoQ also acts as an antioxidant via a redox cycle. During electron transport, Q is reduced to the ubiquinol (QH2) form as it picks up electrons and protons, and is oxidized to the ubiquinone (Q) as it releases electrons and protons. In contrast to other redox cycling antioxidants, CoQ inhibits both the initiation and the propagation of lipid and protein oxidation. Multiple studies have shown that CoQ is beneficial in prevention and treatment of cardiovascular diseases, including hypertension, angina and heart failure [12–14]. In this context, we hypothesized that CoQ supplementation in patients with PAH would improve mitochondrial energetic and synthetic functions, and potentially benefit patients. To test this hypothesis, we evaluated blood levels of reduced and oxidized CoQ, hemoglobin and RDW, metabolic parameters of redox status including reduced and oxidized glutathione and pyruvate and lactate, in PAH patients in comparison to healthy controls at baseline and following 12 weeks of oral CoQ supplementation.

Materials and methods

Study population

Healthy individuals and patients diagnosed with Class I PAH according to the revised clinical classification of pulmonary hypertension [15] between the ages of 18 and 60 were enrolled. All subjects enrolled with PAH were on stable medications and a diagnostic right heart catheterization had previously confirmed diagnosis. Exclusion criteria included: participation in other studies, hepatic insufficiency (transaminase levels levels > 4 fold the upper limit of normal, bilirubin > 2 fold the upper limit for normal), renal insufficiency (creatinine level > 2.0 mg per deciliter), pregnancy, breastfeeding or lack of use of safe contraception, acute heart failure episode within the last 3 months, known allergy to any of the study drugs, history of drug or alcohol abuse within the last 12 months. All patients provided written informed consent under an Institutional Review Board approved protocol at the Cleveland Clinic.

CoQ therapy

Individuals received treatment for 12-weeks with 100 mg capsules of Co-enzyme Q10 (the reduced form, Lot# 07319060 and Lot# 31111, Health Thru Nutrition, Westbury, NY 11590) taken orally three times a day for a total daily dose of 300 mg. This formulation was chosen because it is both hydrosoluble and liposoluble. Individuals were evaluated clinically and for research testing at enrollment to the study (time 0), and at 6 and 12 weeks after starting the treatment. CoQ tablets were brought to each visit and counted by investigators in order to monitor adherence to dosage and ingestion. Furthermore, total and reduced CoQ levels were measured in blood at each visit by Mayo Medical laboratories using high performance liquid chromatography with electrochemical detection [16].

Clinical assessment of PAH: pulmonary vascular and cardiac function

Each PAH patient was classified by the New York Heart Association functional status (I–IV) based on symptoms and severity. Functional status was also assessed by a standard 6 minute walk which was performed in accordance with the American Thoracic Society Recommended guidelines, which included assessment of dyspnea and fatigue by the Borg Dyspnea and Fatigue Scale [17] B-type natriuretic peptide (BNP) at each visit provided quantitative assessment of acute heart failure.

Echocardiograms were performed at baseline and week 12 for all PAH patients as previously described [18,19]. Two dimensional echocardiogram and Doppler exams were performed by a single experienced sonographer, on a General Electric Healthcare Vivid E9 4D Imaging System, Wauwatosa, WI. Interventricular septal (IVSD) thickness in end-diastole, left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD) and posterior wall thickness (PWD) in diastole were measured from the 2D parasternal long axis image following American Society of Echocardiography (ASE) guidelines [18,19]. Left ventricular (LV) mass was determined from 2D measurements using the formula: LV Mass$= 1.04(IVS + PW + LVEDD)^3 - LVEDD^3) - 13.6g$ (Devereaux Regression) LV ejection fraction was determined by visual assessment, and/or apical biplane volumes. LV end-diastolic and end-systolic volumes were calculated from the apical 4 and 2 chamber views using the modified Simpson method. LV fractional shortening was determined from parasternal 2D analysis (LVEDD–LVESD/LVEDD $\times$ 100). Right ventricular (RV) end-diastolic and end-systolic areas were measured in the apical 4-chamber view by tracing the endocardial border of the RV and the tricuspid annular plane. RV fractional area change was calculated as follows: RV end-diastolic area minus RV end-systolic area divided by RV end-diastolic area $\times$ 100. Right atrial volume was measured in the apical 4-chamber view by using the single-plane area–length method. The peak pulmonary artery systolic pressure (PASP) was estimated from the systolic pressure gradient between the RV and the right atrium by the peak continuous-wave Doppler velocity of the TR jet using the modified Bernoulli equation plus estimated right atrial pressure (RAP). RAP was estimated from the subcostal window approach measuring changes in inferior vena caval size and collapsibility as determined by the respiratory sniff test following ASE guidelines. Echo-Doppler was used to estimate pulmonary vascular resistance (PVR). The highest Doppler

Table 1
Baseline characteristics of study population.

Variable	Healthy, $N = 7$	PAH, $N=8$	P-value
Age (years)	42 ± 5	41 ± 3	0.8
Race (Caucasian/African American/Asian)	6/0/1	7/1/0	0.2
Gender (female/male)	6/1	7/1	0.9
Height (cm)	178 ± 7	167 ± 4	0.3
Weight (kg)	62 ± 5	82 ± 8	0.04
Pulse (beats/min)	64 ± 3	81 ± 6	0.02
O_2 saturation (% of hemoglobin)	99.3 ± 0.3	97.6 ± 0.5	0.02
RVSP (mmHg)[a]	–	67 ± 7	–
6 minute walk distance (M)	–	490 ± 28	–
BNP (pg/ml)[b]	–	57 ± 34	–
Fick Cardiac Index (l/min)[a]	–	2.5 ± 0.3	–
Mean pulmonary arterial pressure (mmHg)[a]	–	49 ± 5	–
Pulmonary vascular resistance[a] (wood units)	–	9.9 ± 2	–
NYHA classification (I/II/III/IV)	–	0/7/1/0	

[a] Right heart catheterization done as diagnostic procedure not for research; RVSP, Right Ventricular Systolic Pressure.
[b] BNP, B-type Natriuretic Peptide.

continuous wave tricuspid valve peak velocity jet obtained from multiple views (parasternal long axis, parasternal short axis, apical 4-chamber, subcostal or apical off-axis imaging) was determined
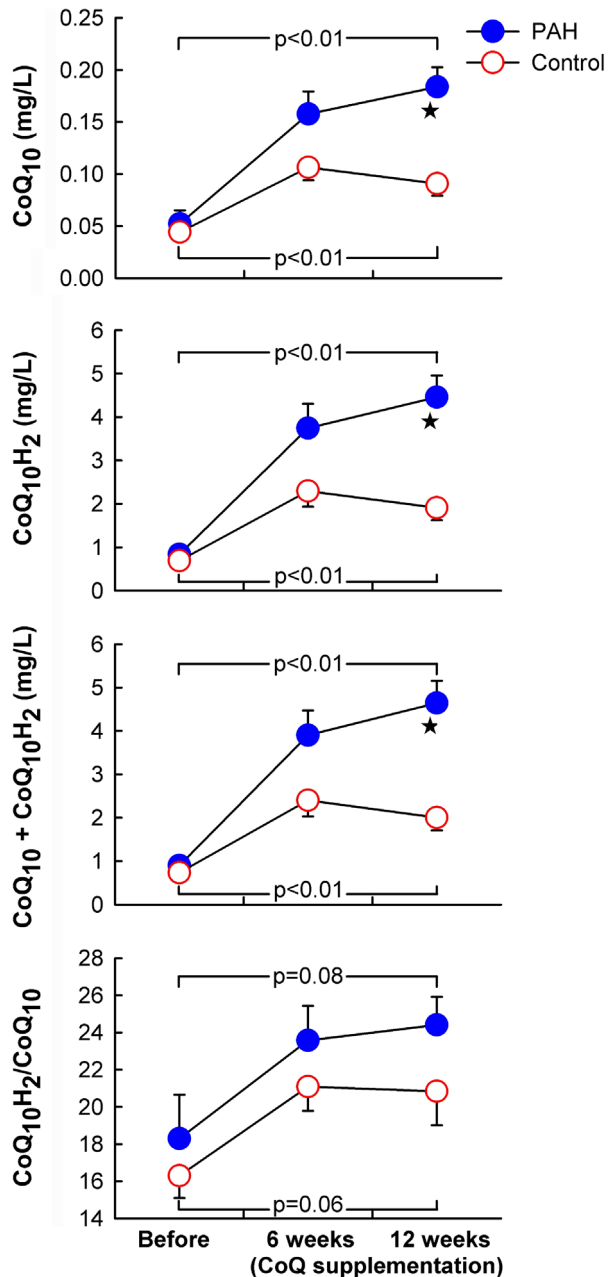
Fig. 1. CoQ supplementation increases serum levels of the oxidized ubiquinone (CoQ$_{10}$) and the reduced ubiquinol (CoQ$_{10}$H$_2$). PAH patients have greater increase in CoQ than controls at 12 weeks. ★ indicates $P < 0.05$ comparison of PAH to healthy control at the indicated time. The brackets at the top and bottom of each panel show P values for the ANOVA/post-hoc Tukey test within PAH or control groups, respectively.

as the maximum tricuspid regurgitant velocity (TRV). Pulsed wave Doppler sample was placed in the right ventricular outflow tract (RVOT) at the level of the aortic valve in the parasternal short axis view just below the pulmonic valve so that pulmonic valve closure is identified. The Doppler spectrum was traced to determine the time velocity integral of the RVOT. PVR was estimated by: PVR (Wood units)$=$TRV$_{MAX}$ (m/s)/RVOT$_{TVI}$ (cm) $\times 10 + 0.16$. Tricuspid annular plane systolic excursion was obtained from the apical 4 chamber RV focused view with M-mode echocardiography across the TV annulus, measuring the distance of longitudinal annular movement from end diastole to end systole towards the apex. Pulsed Doppler cardiac time intervals of right ventricular ejection time (RVET) and Tricuspid valve opening to closure were used to calculate RV myocardial performance or TEI Index values. With the exception of the TR continuous wave maximum velocity all echo parameters were measured in triplicate and reported as an average value.

Metabolic effects of CoQ

To assess CoQ effects on intracellular metabolism and redox status, plasma levels of lactate and pyruvate, reduced and oxidized glutathione (GSH, GSSG, respectively), and the reduced (CoQ$_{10}$H$_2$) and oxidized (CoQ) forms of CoQ were determined at each visit. Plasma lactate and pyruvate measurements were performed in diluted samples using the Lactate Assay K607-100 and Pyruvate Assay K609-100, respectively (BioVision, Inc., Milpitas, CA). A range of authentic lactate (0–0.25 mM) and pyruvate (0–0.1 mM) standards were used in the assay. Reduced and oxidized glutathione were measured in serum [20]. In brief, total glutathione levels were determined by mixing equal amounts of serum with 10 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) in 100 mM potassium phosphate, pH 7.5, which contained 17.5 μM EDTA. An aliquot (50 μl) of the solution was added to a cuvette containing 0.5 U of glutathione disulfide reductase (Sigma type III, Sigma Chemical, St. Louis, MO) in 100 mM potassium phosphate and 5 mM EDTA, pH 7.5. After 1 min, the reaction was initiated with 220 nmol of NADPH in a final reaction volume of 1 ml. The rate of reduction of DTNB was recorded continuously at 412 nm by a spectrophotometer with a Kinetics/Time feature (Beckman DU-640, Beckman Instruments, Inc., Fullerton, CA). In order to assay GSSG, equal volumes of serum and N-ethylmaleimide (NEM) were added to serum. An aliquot (50 μl) of the solution was passed at one drop/sec through a C15 Sep-Pak cartridge (Walters Associates, Framingham, MA) that had been washed with methanol followed by water. The cartridge was then washed with 1 ml of 100 mM potassium phosphate and 5 mM EDTA, pH 7.5. A 750 μl aliquot of the eluate was added to a cuvette with 250 nmol of DTNB and 0.5 U of GSSG reductase. The assay then proceeded as in the measurement of total GSH. The levels of GSH and GSSG were based on standard curves.

Hematologic effects of CoQ: heme synthesis and hematopoiesis

To assess heme synthetic effects of CoQ, blood samples were obtained for measures of complete blood cell count, red

Table 2
CoQ levels in PAH patients and Healthy Controls.

Variable	PAH				Controls			
	Baseline	6 weeks	12 weeks	P-value	Baseline	6 weeks	12 weeks	P-value
Total CoQ (mg/L)	0.89 ± 18	3.9 ± .6	4.6 ± .5	< 0.001	0.73 ± .03	2.4 ± .4	2.0 ± 0.3*	< 0.001
Reduced CoQ (mg/L)	0.84 ± .16	3.7 ± .6	4.4 ± .5	< 0.001	0.69 ± .03	2.3 ± .4	1.9 ± 0.3*	< 0.001

* $P \leq 0.05$ PAH compared to controls for the corresponding time point in the study.

cell distribution width (RDW), and erythropoietin (epo). epo was measured in plasma using quantikine ELISA (R&D system, MN) [21].

Statistical analysis

Data are reported in aggregate as mean $\pm$ SEM for continuous data and as n (%) for categorical data. Statistical changes from
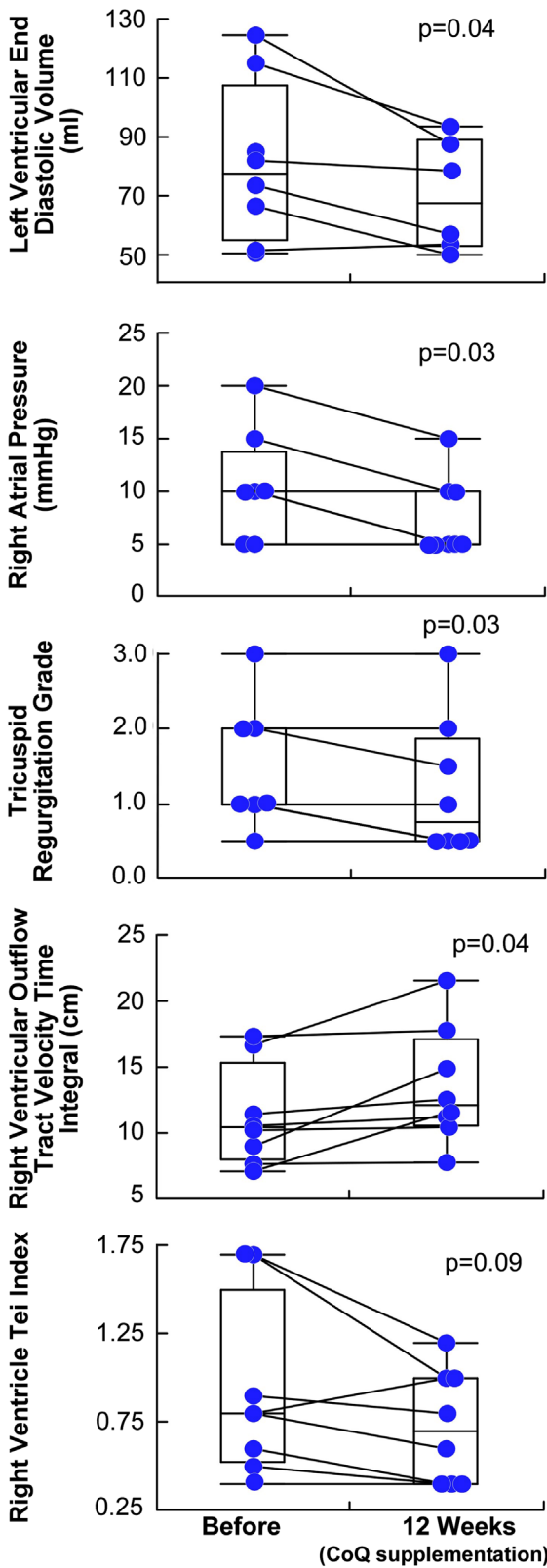

Fig. 2. Cardiac function improves in PAH patients receiving CoQ. Boxes show the median and 25% and 75%. Paired samples from baseline to 12 weeks of CoQ are connected by lines.
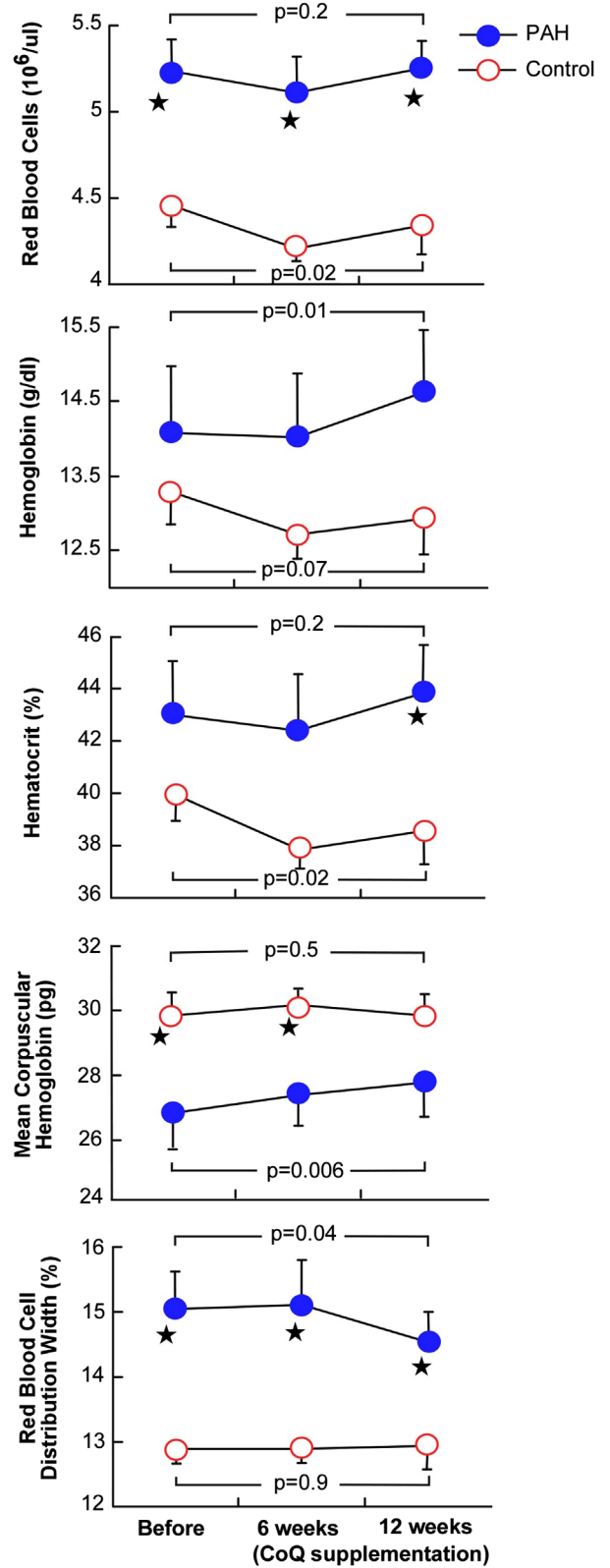
Fig. 3. Hemoglobin and mean corpuscular hemoglobin increase, and RDW decreases in PAH patients supplemented with CoQ. Healthy controls have a tendency to decrease hemoglobin, and do not show changes in RDW. ★ indicates $P < 0.05$ comparison of PAH to healthy controls at the indicated time.

baseline were assessed using a paired *t*-test. Spearman correlation coefficients were used to detect associations between parameters including all visits. Significance was set at an alpha of 0.05. Data were analyzed using the JMP 9 statistical software (SAS Institute, Cary, NC). For Figs. 1, 4 and 5 the data are reported as mean ± SEM. ANOVA was used to analyze the data within PAH or control groups. The Tukey test was used when differences were detected by ANOVA. If the data were not normally distributed, a log transform was used for statistical analysis only. Comparisons between PAH and healthy controls at each time point were carried out using *t*-test.

Results

Study population

Ten patients with PAH (9 idiopathic and 1 heritable) and 8 healthy controls were enrolled. Two PAH patients dropped out of the study. One after 1 week in the study due to unremitting headache, and another after 6 weeks of CoQ due to hyperkalemia. One healthy control dropped out after 1 week of CoQ due to nausea. PAH patients were similar to healthy subjects in terms of age, gender, race and height, but tended to have greater body mass, and higher resting heart rate and lower oxygen saturation (Table 1). All PAH subjects had no change in their PAH therapy during the study. Two PAH subjects were on a short course of antibiotics between visit 2 and 3 and one PAH subject had a 5 day course of prednisone for a gout flare. All subjects maintained the same level of activity during the course of the study.

CoQ levels increase in healthy controls and PAH patients with supplementation

PAH and controls had equivalent endogenous total and reduced CoQ levels ($P=0.4$). Following supplementation, for PAH patients, total and reduced CoQ increased ~4-fold at week 6, and ~5-fold at week 12. In controls, total and reduced CoQ also increased, but only by ~3-fold at 6 and 12 weeks. Thus, the total, reduced and oxidized CoQ levels were higher in PAH patients than controls at week 12 (Fig. 1 and Table 2). The reduced-to-oxidized CoQ ratio tended to be higher in PAH as compared to control, but did not reach significance ($P=0.1$).

CoQ effects on clinical parameters

Although the primary aim of the study was to determine effects of CoQ on mitochondrial functions, clinical evaluations were performed to detect any potential benefits and/or adverse effects. The 6-minute walk distances and BNP levels did not significantly change with CoQ supplementation (all $P > 0.1$). The Borg Dyspnea and Fatigue scores were similar before and after CoQ supplementation (all $P > 0.4$). However, the Borg Dyspnea and Fatigue scores were directly correlated to CoQ levels prior to supplementation (Dyspnea and CoQ, $R^2=0.59$, $P=0.026$; Fatigue and CoQ, $R^2=0.92$, $P=0.0005$) and the relationships were lost when patients were receiving CoQ (all $P > 0.3$). Echocardiographic parameters improved over the course of the study (Fig. 2). The left ventricular end diastolic volume (LVEDV) decreased significantly [LVEDV (ml): baseline 81 ± 10, 12 weeks CoQ 70 ± 8; $P=0.04$]. The right ventricular outflow (velocity time interval) improved [baseline 11.3 ± 1.4 cm, 12 weeks CoQ 13.5 ± 1.6 cm; $P=0.04$], and RV myocardial performance (Tei index) tended to improve [RV Tei index: baseline 0.9 ± 0.2, 12 weeks CoQ 0.7 ± 0.1; $P=0.09$] (Fig. 2). In addition, the right atrial pressure (RAP) decreased with CoQ and tricuspid regurgitation (TR) decreased [RAP (mmHg): baseline 10 ± 2, 12 weeks CoQ 8 ± 1; $P=0.03$ and TR grade: baseline 1.4 ± 0.3, 12 weeks CoQ 1.2 ± 0.3; $P=0.03$] (Fig. 2). There were no significant correlations between echocardiographic parameters and CoQ (all $P > 0.1$).

CoQ effects on heme synthesis in PAH and controls

Hemoglobin (Hgb) and mean corpuscular Hgb (MCH) increased in PAH patients, but the red blood cell count and hematocrit remained unchanged (Fig. 3 and Table 3). In parallel, the RDW decreased in PAH patients with CoQ (Table 3). Hemoglobin and MCH were unrelated to total CoQ, but were directly related to the reduced-to-oxidized CoQ ratio at baseline (Hgb relation to QH_2/Q, $R^2=0.57$, $P=0.02$; MCH relation to QH2/Q, $R^2=0.54$, $P=0.03$) and tended to be directly related after 12 weeks of CoQ supplementation (Hgb relation to QH2/Q, $R^2=0.27$, $P=0.18$; MCH relation to QH2/Q, $R^2=0.45$, $P=0.06$). These data indicate that hemoglobin synthesis at baseline is limited by CoQ, even though levels are in the normal range. In contrast to PAH patients, but similar to prior reports, red blood cell numbers and hematocrit decreased slightly, and mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin content and RDW did not change in healthy controls on CoQ (all $P > 0.1$) (Table 3). Erythropoietin (Epo) levels in patients with PAH are higher than in healthy controls [21]. Here, Epo levels were measured to assess other possible mechanisms of CoQ effect on hemoglobin production. Epo levels in PAH patients at baseline were similar to levels with CoQ supplementation [Epo (mIU/ml): baseline 20.8 ± 5.4, 6 weeks 18.3 ± 4.5, 12 weeks 19.5 ± 2.8; $P=0.8$].

Effects of CoQ on mitochondria metabolic function

Serum lactate and pyruvate levels were measured as biomarkers of mitochondrial-metabolism. Over time of CoQ supplementation, lactate did not change within control group, but PAH patients had significantly higher lactate than controls by week

Table 3
Hematologic effects of CoQ in PAH and healthy controls.

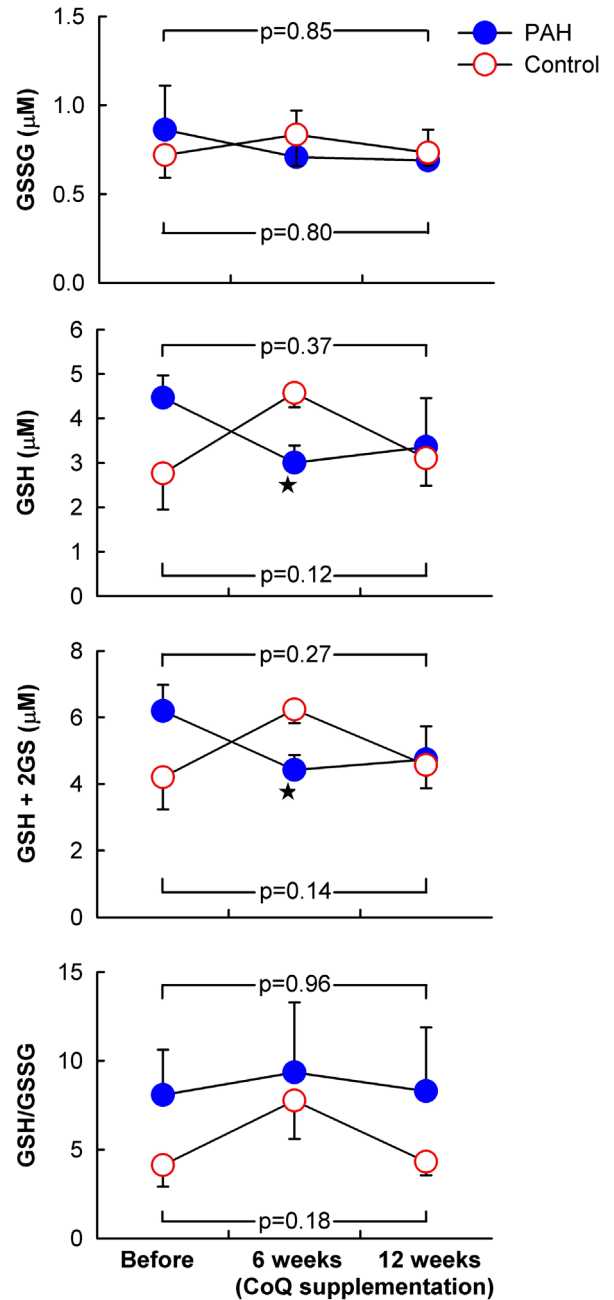| Variable	PAH				Controls			
	Baseline	6 weeks	12 weeks	*P*-value	Baseline	6 weeks	12 weeks	*P*-value
Red blood cell (m/μl)	5.2 ± 0.2*	5.1 ± 0.2*	5.2 ± 0.2*	0.2	4.5 ± 0.1	4.2 ± 0.1	4.3 ± 0.2	0.02
Hematocrit (%)	43.0 ± 2.1	42.4 ± 2.1*	43.8 ± 1.8*	0.2	39.9 ± 1.0	37.9 ± 0.7	38.6 ± 1.3	0.02
Hemoglobin (g/dl)	14.1 ± 0.9	14.0 ± 0.9	14.6 ± 0.8	0.01	13.3 ± 0.4	12.7 ± 0.3	12.9 ± 0.5	0.07
Mean corpuscular volume (fl)	82.1 ± 2.4	83.0 ± 2.2	83.5 ± 2.0	0.06	89.7 ± 1.3	90.0 ± 1.0	88.9 ± 1.3	0.3
Mean corpuscular hemoglobin (pg)	26.8 ± 1.1	27.3 ± 0.9	27.8 ± 1.0	0.006	29.9 ± 0.8	30.2 ± 0.5	29.9 ± 0.7	0.5
Mean corpuscular hemoglobin content (g/dl)	32.6 ± 0.6	33.0 ± 0.4	33.2 ± 0.7*	0.06	33.3 ± 0.5	33.6 ± 0.3	33.5 ± 0.4	0.6
Red blood cell distribution width (%)	15.0 ± 0.6*	15.1 ± 0.7*	14.5 ± 0.5*	0.04	12.9 ± 0.2	12.9 ± 0.2	12.9 ± 0.4	1.0

* $P \leq 0.05$ PAH compared to controls for the corresponding time point in the study.
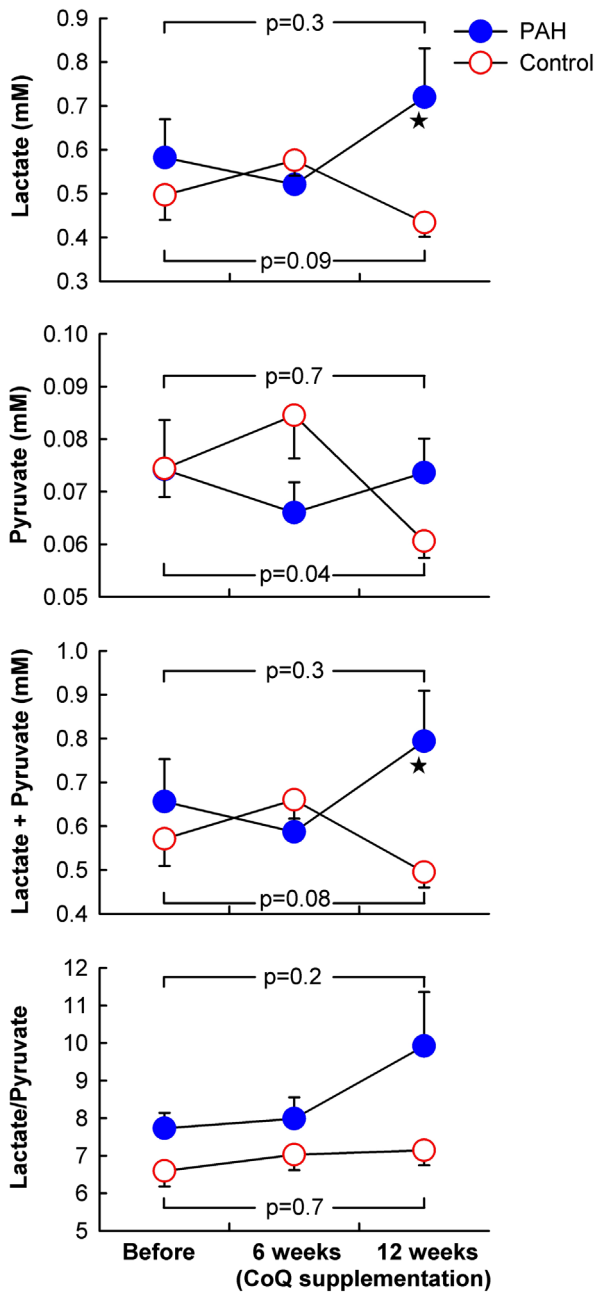
Fig. 4. Lactate and pyruvate do not change with CoQ supplementation within PAH or healthy control groups with CoQ. The lactate levels for PAH patients at 12 weeks is significantly higher than controls. ★ indicates $P < 0.05$ comparison of PAH to healthy control at the indicated time. The brackets at the top and bottom of each panel show P values for ANOVA/Tukey' post-hoc test within PAH or control groups, respectively.

Fig. 5. Reduced GSH and GSSG do not change with CoQ supplementation in PAH or healthy controls. ★ indicates $P < 0.05$ comparison of PAH to healthy control at the indicated time. The brackets at the top and bottom of each panel show P values for ANOVA within PAH or control groups, respectively.

12 (Fig. 4). Pyruvate levels changed over time in controls ($P=0.04$), but there was no detectable change in pyruvate levels in PAH patients over time of CoQ supplementation (Fig. 4). The net effect was that total lactate+pyruvate levels were higher in PAH than control patients at 12 weeks. The lactate/pyruvate ratios did not change with CoQ supplementation in the PAH or control groups, and there were no detectable differences between the groups at any time (Fig. 4). Lactate and pyruvate levels were not correlated with CoQ (all $P > 0.1$).

Reduced (GSH) and oxidized (GSSG) glutathione were measured as another redox status indicator. GSSG levels did not change significantly in PAH patients or controls (all $P > 0.8$). Reduced (GSH) and total (GSH+GSSG) levels were significantly lower in PAH patients as compared to Controls at the 6 week time point. However, PAH patients and Controls had statistically indistinguishable GSH and total GSH+GSSG by week 12 of CoQ supplementation. The GSH/GSSG ratio did not change significantly over time of CoQ supplementation for PAH or control subjects, and there was no statistically detectable difference in the ratios between PAH and control individuals at any time point (Fig. 5).

Discussion

CoQ is essential to key mitochondria functions. Oxidative phosphorylation by the mitochondria requires CoQ. Complex I and complex II in the inner mitochondrial membrane transfer

electrons to CoQ, which subsequently transfers the electrons to Complex III. CoQ also provides antioxidant function, redox cycling from the reduced ubiquinol (QH2) to the oxidized ubiquinone (Q) as it transfers electrons in the mitochondria. Furthermore, CoQ is an obligate cofactor for mitochondria uncoupling, which reduces the electrochemical gradient, and lowers the capacity to produce ATP as well as reactive oxygen species [23]. Increased mitochondria production of reactive oxygen species are well-described in PAH and contribute to the vascular remodeling [24,25]. Thus, CoQ supplementation theoretically might improve cellular metabolism and minimize oxidative damage. To our knowledge, this is the first study to investigate CoQ effects on mitochondrial dysfunction in patients with PAH. Similar to prior reports of positive effects of CoQ on myocardial metabolism and function in left heart failure [2,12], CoQ improved the right and left heart function in PAH patients in this study. However, the greatest effect of CoQ occurred in the nonmetabolic mitochondrial function of heme synthesis in the 12 week period of the study.

Pulmonary hypertension in avian species, rodent species, lambs and humans is characterized by deficiencies in mitochondrial functions [2,22,24–29]. For example, endothelial cells in the human PAH lung have mitochondrial respiration abnormalities, and have greater reliance on glycolysis [2]. Consistent with this finding, lungs and hearts of patients with PAH have greater uptake of the glucose analog tracer [^{18}F] fluoro-deoxy-D-glucose (FDG) by positron emission tomography (PET) [2,30]. Dichloroacetate (DCA), which inhibits pyruvate dehydrogenase kinase (the enzyme that selectively blocks pyruvate dehydrogenase and the entry of pyruvate in the Tricarboxylic Acid cycle), reverses pulmonary hypertension of Fawn Hooded rats [26]. Here, measures of serum pyruvate, lactate and glutathione did not change in PAH or control subjects over time of the study. This may be due to the dose of the CoQ or the 12 week duration of the supplementation, either of which may be inadequate to see effects in serum levels of metabolic indices.

Many studies have identified abnormalities in iron metabolism and hematologic abnormalities in PAH [6–9,22]. Consistent with the concept of defective heme production, PAH patients here, and in past studies, have greater variation in the size distribution of the red cell distribution width (RDW), which directly correlates to pulmonary artery pressures and inversely to 6-minute walk distances [6–9]. Unfortunately, iron levels, transferrin, or B12 levels which impact hemoglobin synthesis, were not available for participants in the study, and this is a limitation for the study. However, in this study, hemoglobin and mean corpuscular hemoglobin increased in the PAH patients, identifying a role for CoQ in heme production and red blood cell maturation. Although, standard clinical biomarkers, such as the 6-minute walk distance and BNP did not improve, echocardiographic measures of right and left heart function improvements provided promise that improvements may have occurred if CoQ therapy were continued for a longer time. In fact, findings of unchanged exercise capacity with short-term CoQ supplementation in heart failure have been documented in prior studies [14]. In a randomized control study of CoQ (300 mg daily) in 420 patients with NYHA class 3 or 4 heart failure [23], BNP tended to decrease after 3 months of CoQ. However, striking clinical outcomes were seen after 2 years of CoQ, e.g. improvement in NYHA class, 50% reduction in major adverse cardiovascular events including death, and 50% drop in all cause mortality. CoQ levels have been shown, in fact, to be an independent predictor of mortality in patients with congestive heart failure; the lower the plasma CoQ levels, the greater the risk of mortality from congestive heart failure. The association between increased mortality in CHF and lower CoQ levels is stronger than the association observed between CHF mortality and the well-established NT-proBNP [31]. Although CoQ levels are higher in the heart than in other organs in the body, CoQ levels are deficient in myocardium of patients with congestive left-sided heart failure [32]. In this context, although PAH and controls had similar endogenous CoQ levels at baseline, supplementation led to greater increase of CoQ levels in PAH patients as compared to healthy controls. Adherence may have been better in the PAH group, but more likely the results indicate that metabolism of CoQ is altered in the patients [14].

This mechanistic study of CoQ effects in PAH did not aim to test clinical improvement. Standard clinical markers of 6-minute walk and BNP did not change over the 12 weeks of the study. However, the decrease in RDW and improvements in echocardiographic parameters at 12 weeks suggest that trials of longer duration, and/or higher dose of CoQ, may demonstrate benefit. Equally important, although this was a small cohort, CoQ was well tolerated, confirming minimal side effects of supplementation [32]. Altogether, the known relationship of CoQ to outcomes in left-sided heart failure patients, and the finding that CoQ supplementation improves heme synthetic and cardiac functions in PAH, provide rationale for studies of longer duration to evaluate clinical benefits over time.

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