STUDIES OF HYPOXEMIC/ REOXYGENATION INJURY: WITHOUT AORTIC CLAMPING

VII. Counteraction of oxidant damage by exogenous antioxidants: Coenzyme Q₁₀

Coenzyme Q₁₀ (CoQ₁₀) is a natural mitochondrial respiratory chain constituent with antioxidant properties. This study tests the hypothesis that CoQ₁₀ administered before the onset of reoxygenation on cardiopulmonary bypass, can reduce oxygen-mediated myocardial injury and avoid myocardial dysfunction after cardiopulmonary bypass. The antioxidant properties of CoQ₁₀ were confirmed by an *in vitro* study in which normal myocardial homogenates were incubated with the oxidant, *t*-butylhydroperoxide. Fifteen immature piglets (<3 weeks old) were placed on 60 minutes of cardiopulmonary bypass. Five piglets underwent cardiopulmonary bypass without hypoxemia (oxygen tension about 400 mm Hg). Ten others became hypoxemic on cardiopulmonary bypass for 30 minutes by lowering oxygen tension to approximately 25 mm Hg, followed by reoxygenation at oxygen tension about 400 mm Hg for 30 minutes. In five piglets, CoQ₁₀ (45 mg/kg) was added to the cardiopulmonary bypass circuit 15 minutes before reoxygenation, and five others were not treated (no treatment). Myocardial function after cardiopulmonary bypass was evaluated from end-systolic elastance (conductance catheter), oxidant damage (lipid peroxidation) was assessed by measuring conjugated diene levels in coronary sinus blood, and antioxidant reserve capacity was determined by measuring malondialdehyde in myocardium after cardiopulmonary bypass incubated in the oxidant, *t*-butylhydroperoxide. Cardiopulmonary bypass without hypoxemia caused no oxidant damage and allowed complete functional recovery. Reoxygenated hearts (no treatment) showed a progressive increase in conjugated diene levels in coronary sinus blood after reoxygenation (2.3 ± 0.6 A233 nm/0.5 ml plasma at 30 minutes after reoxygenation) and reduced antioxidant reserve capacity (malondialdehyde: 1219 ± 157 nmol/g protein at 4.0 mmol/L *t*-butylhydroperoxide), resulting in severe postbypass dysfunction (percent end-systolic elastance = 38 ± 6). Conversely, CoQ₁₀ treatment avoided the increase in conjugated diene levels (2.1 ± 0.6 vs 1.1 ± 0.3 , $p < 0.05$ vs no treatment), retained normal antioxidant reserve (896 ± 76 nmol/g protein, $p < 0.05$ vs no treatment), and allowed nearly complete recovery of function ($94\% \pm 7\%$, $p < 0.05$ vs no treatment). We conclude that reoxygenation of the hypoxemic immature heart on cardiopulmonary bypass causes oxygen-mediated myocardial injury, which can be limited by CoQ₁₀ treatment before reoxygenation. These findings imply that coenzyme Q₁₀ can be used to surgical advantage in cyanotic patients, because therapeutic blood levels can be achieved by preoperative oral administration of this approved drug. (J THORAC CARDIOVASC SURG 1995; 110:1221-7)

Kiyozo Morita, MD, Kai Ihnken, MD, Gerald D. Buckberg, MD, and
Helen H. Young, PhD, *Los Angeles, Calif.*

From the Division of Cardiothoracic Surgery, Department of
Surgery, University of California at Los Angeles School of
Medicine, Los Angeles, Calif.

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Address for reprints: Gerald D. Buckberg, MD, UCLA Medical
Center, Department of Surgery, Division of Cardiothoracic
Surgery, B2-375 CHS, 10833 Le Conte Ave., Los Angeles, CA
90024-1741.

Repair of cyanotic heart defects by the use of cardiopulmonary bypass (CPB) is becoming more frequent in infants and newborns.¹ Postoperative myocardial dysfunction is, however, the major cause of morbidity and mortality despite successful surgical correction and is more common in infants than in adult cardiac patients.²

Conventionally, CPB is initiated in previously hypoxemic patients at hyperoxemic oxygen tension (P_{O_2}) levels (i.e., 300 to 400 mm Hg) and may result in an "unintended reoxygenation injury" without considering the deleterious effects of hyperoxemia. Recent clinical findings document that chronic cyanosis depletes endogenous antioxidants in myocardium,³ whereas hyperoxemic CPB per se can generate oxygen free radicals.⁴ We have speculated that such abrupt reoxygenation causes an unintended oxygen-mediated myocardial injury that adds to subsequent intraoperative ischemic injury and causes myocardial dysfunction after bypass.^{5,6} This hypothesis is supported by clinical reports that show evidence of myocardial lipid peroxidation in preischemic biopsy samples from cyanotic hearts,⁷ inadequate cardioplegic protection in cyanotic patients,⁸ and myocardial dysfunction in infants placed on extracorporeal membrane oxygenation support despite absence of surgical ischemia.⁹ We have shown recently that myocardial dysfunction associated with lipid peroxidation occurs after reoxygenation of hypoxemic immature piglet hearts on the initiation of CPB, which could be ameliorated by the antioxidants, *N*-(2-mercaptopropionyl)-glycine and catalase.¹⁰ Unfortunately, these antioxidants are not available clinically.

Coenzyme Q_{10} (CoQ_{10}) is a natural constituent of the mitochondrial respiratory chain that plays an important role in the regulation of mitochondrial oxidative phosphorylation.¹¹ In addition, the antioxidant property of CoQ_{10} has been shown in vitro¹² and in vivo.^{13,14} This study tests the hypothesis that CoQ_{10} can reduce in vitro lipid peroxidation in myocardial homogenates exposed to the oxidant *t*-butylhydroperoxide and limit reoxygenation injury of in vivo hypoxemic immature heart.

Material and methods

Preparation. Twenty immature, 2- to 3-week-old Duroc-Yorkshire piglets (3 to 5 kg) were premedicated with 0.5 mg/kg diazepam intramuscularly, anesthetized with 30 mg/kg pentobarbital intraperitoneally followed by 5 mg/kg intravenously each hour, and the lungs ventilated on a volume-limited respirator (Servo 900D, Siemens-Elema,

Solna, Sweden) via a tracheostomy. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences, published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). The surgical preparation for CPB, including vessel cannulation for blood sampling and monitoring, is similar to that described previously.⁶

Experimental protocols

***In vitro* lipid peroxidation in myocardial homogenates.** To evaluate the antioxidant properties of CoQ_{10} and define the appropriate CoQ_{10} dose, myocardial tissue from five piglets that were anesthetized and observed for 5 hours was analyzed. They were not subjected to CPB or hypoxemia/reoxygenation, and samples were homogenized and incubated with 2 mmol *t*-butylhydroperoxide and CoQ_{10} (0, 0.1, 0.2, and 0.4 mm) for 30 minutes at 37° C. The formation of thiobarbituric acid-reactive substances, an index of lipid peroxidation, was measured spectrophotometrically at 532 nm and a reaction curve was generated. A malondialdehyde standard curve was run simultaneously, and lipid peroxidation was expressed as nanomoles malondialdehyde per gram protein. The effects of CoQ_{10} at each concentration was assessed by comparison with malondialdehyde production without CoQ_{10} in parallel studies (control).

Nonhypoxemic control

***In vivo* hypoxemia-reoxygenation study.** Five normoxemic animals were anesthetized, instrumented, and observed over 5 hours to provide baseline (control) functional and biochemical data without CPB.

Five other piglets underwent 60 minutes of CPB without hypoxemia, followed by 30 minutes of observation after CPB to determine the effects of CPB per se (CPB group).

Experimental. All studies were performed in piglets on CPB. In 10 piglets, 30 minutes of hypoxemia was produced on CPB by adding N_2 in the gas mixture in the CPB circuit to reduce alveolar oxygen tension (P_{aO_2}) to approximately 25 mm Hg. CPB flow rate was raised to maintain mean aortic pressure at 50 mm Hg and to avoid systemic acidosis. This hypoxemic interval was followed by 30 minutes of reoxygenation on CPB at P_{O_2} approximately 400 mm Hg.

No treatment. Five piglets were reoxygenated without CoQ_{10} .

CoQ_{10} treatment. Five piglets were treated with CoQ_{10} (45 mg/kg) added to the CPB circuit 15 minutes before reoxygenation. This dose was selected to achieve the 0.2 mmol blood concentration shown in the in vitro studies and accounted for the piglet blood volume and the dilution produced by the extracorporeal circuit volume.

Final functional assessments were performed and specimens for biochemical analyses were obtained after 30 minutes of observation after CPB.

Measurements

Hemodynamics. Cardiac output was determined by duplicate injections of 1 ml of 4° C saline solution into a

central venous catheter. Cardiac index (CI), systemic vascular resistance index (SVRI), and left ventricular stroke work index (LVSWI) were calculated with the following equations:

$$CI \text{ (ml/min/kg)} = CO / \text{body weight (kg)}$$

$$SVRI \text{ (mm Hg} \cdot \text{min} \cdot \text{L}^{-1} \cdot \text{kg)} = (\text{MAP} - \text{CVP})$$

$$(\text{mm Hg}) \cdot CO^{-1} \text{ (ml/min)} \cdot BW \text{ (kg)}$$

$$LVSWI \text{ (g} \cdot \text{m/kg)} = (\text{MAP} - \text{LAP})$$

$$\times CO \times 0.0136 / (\text{HR} \times \text{body weight [kg]})$$

where MAP is mean aortic pressure, LAP is mean left atrial pressure, CVP is central venous pressure, and CO is cardiac output in liters per minute.

Left ventricular contractility. Left ventricular (LV) pressure and conductance catheter signals were amplified and digitalized to inscribe LV pressure-volume loops. After the procedure for correction of parallel conductance, a series of pressure-volume loops under variable loading conditions was generated by rapid transient occlusion of the inferior vena cava during a 7-second period of apnea, at a control condition, and 30 minutes after CPB was discontinued.¹⁵

The end-systolic pressure volume relationship was analyzed by an interactive videographics data analysis computer program (Spectrum, Bowman Gray School of Medicine, Winston-Salem, N.C.) on an 386/33 MHz IBM computer (IBM, Armonk, N.Y.), and LV performance was described as the slope of linear regression (end-systolic elastance [Ees]), as described previously.¹⁶ Post-bypass LV contractility was assessed by percent recovery of prebypass control value (percent Ees).

Myocardial function was also evaluated by infusion of blood from the CPB circuit at 10 ml/min/kg over a 3-minute period before and after CPB to inscribe Starling function curves as described previously.⁶

Myocardial tissue injury from oxidants

Myocardial conjugated diene production. During hypoxemia and reperfusion, blood samples were taken from coronary effluent (coronary sinus blood) to assess changes in conjugated diene levels. Blood samples were centrifuged immediately for 5 minutes at 1000g, and plasma (0.5 ml) was then stored in liquid nitrogen for later measurement by the method of Lesnfsky and coworkers.¹⁷

Antioxidant reserve capacity. The myocardial endogenous antioxidant state was assessed by determining in vitro lipid peroxidation in cardiac tissues¹⁸ after completion of each in vivo experiment by the method of Godin and coworkers¹⁸ to assess the vulnerability to subsequent oxidant stress.

Statistics. Data were analyzed with the use of the StatView V2.0 program (Abacus Concepts Inc., Berkeley, Calif.) on a Macintosh IICI computer (Apple, Inc., Cupertino, Calif.). Analysis of variance was used for intergroup comparison and the paired Student *t* test was used for comparison of variables within experimental groups. Differences were considered significant at the probability

Fig. 1. Effect of CoQ₁₀ on *t*-butylhydroperoxide-induced lipid peroxidation of myocardial homogenates. Control: myocardial homogenates incubated without CoQ₁₀. Histogram shows percent malondialdehyde production of control when adding CoQ₁₀ to homogenates. **p* < 0.05 vs control. Note: axis of histogram starts at 50% of control.

level of *p* < 0.05. Group data were expressed as mean plus or minus standard error of the mean.

Results

In vitro lipid peroxidation. Intact myocardial homogenates were exposed to the oxidant, *t*-butylhydroperoxide (2 mmol/L) to induce lipid peroxidation with varying concentrations of CoQ₁₀ (0, 0.1, 0.2, and 0.4 mmol/L). For comparison, malondialdehyde elution without CoQ₁₀ was considered 100%. The addition of CoQ₁₀ to the myocardial homogenates suppressed malondialdehyde production by 18% to 25% in a dose-dependent fashion (Fig. 1).

In vivo hypoxemia-reoxygenation study

Hemodynamics. Systemic (CPB) flow averaged 100 ml/min/kg in control studies without hypoxemia (Table). Hypoxemia on CPB caused systemic vasodilatation in all studies, as SVRI fell to approximately 65% below prehypoxemic values. Consequently, perfusion flow rate was increased initially to approximately 150 ml/min/kg during hypoxemia to keep perfusion pressure approximately 50 mm Hg and reduced thereafter to maintain that pressure. Systemic pH remained between 7.3 and 7.4 in all studies, and no supplemental NaHCO₃ was needed. CoQ₁₀ supplementation of the CPB circuit did not change systemic vascular resistance.

Fig. 2. Postbypass myocardial contractility. LV Ees after discontinuation of CPB, expressed as percent of individual per hypoxemic values. CPB: piglets undergoing CPB without hypoxemia, no treatment: piglets undergoing hypoxemia/reoxygenation without CoQ₁₀ and CoQ₁₀-treated piglets with CoQ₁₀. * $p < 0.05$ vs CPB; † $p < 0.05$ vs no treatment.

Fig. 3. Conjugated diene levels in coronary sinus plasma during hypoxemia and reoxygenation. CPB: piglets undergoing CPB without hypoxemia, no treatment: untreated piglets undergoing hypoxemia/reoxygenation without CoQ₁₀, and CoQ₁₀: piglets treated with CoQ₁₀. * $p < 0.05$ vs CPB and CoQ₁₀; † $p < 0.05$ vs pre-CPB values.

LV performance. CPB per se did not change LVSWI from control levels. In contrast, reoxygenation without CoQ₁₀ reduced LVSWI to $55\% \pm 9\%$ at LAP 10 mm Hg, whereas LVSWI recovered $98\% \pm 14\%$ at comparable LAP in piglets treated with CoQ₁₀ (Table). CPB without hypoxemia (CPB group) allowed complete recovery of Ees (Ees =

Table. Hemodynamic changes before and after CPB

	Before CPB	After CPB	% recovery
Cardiac index (ml/min/kg)			
CPB (no hypoxia)	114 ± 18	82 ± 8	76 ± 10
No treatment	112 ± 8	74 ± 7	69 ± 10
CoQ ₁₀	115 ± 8	104 ± 13*	91 ± 11
Stroke work index (gm · m/kg)			
CPB (no hypoxia)	0.47 ± 0.06	0.55 ± 0.04	117 ± 6
No treatment	0.49 ± 0.06	0.26 ± 0.03	55 ± 9
CoQ ₁₀	0.61 ± 0.03	0.61 ± 0.1*	98 ± 14*

CPB: piglets placed on CPB without hypoxia ($n = 5$), no treatment: hypoxia followed by reoxygenation without CoQ₁₀ ($n = 5$), and CoQ₁₀: CoQ₁₀ 45 mg/kg in CPB circuit 15 minutes before reoxygenation ($n = 5$). * $p < 0.05$ vs no treatment.

98% of pre-CPB values). Conversely, reoxygenation was associated with only $38\% \pm 12\%$ recovery of Ees ($p < 0.05$ vs CPB). The addition of CoQ₁₀ to the CPB prime restored LV contractility to $94\% \pm 12\%$ of pre-CPB levels ($p < 0.05$ vs no treatment) (Fig. 2).

Conjugated dienes. Conjugated diene levels in coronary sinus blood were unchanged in piglets placed on CPB without hypoxemia (Fig. 3). Reoxygenation on CPB (no therapy) raised conjugated diene concentration at 60 minutes on CPB by 64% of pre-CPB value (1.3 ± 0.1 vs 2.3 ± 0.5 , $p < 0.05$), whereas CoQ₁₀ avoided elevation of conjugated diene levels in coronary sinus blood (2.3 ± 0.5 vs 1.2 ± 0.2 , $p < 0.05$).

Antioxidant reserve capacity

Reoxygenation (no treatment) reduced antioxidant reserve capacity, because more malondialdehyde was produced in myocardial homogenates incubated with the oxidant *t*-butylhydroperoxide. Conversely, antioxidant reserve capacity remained normal when CoQ₁₀ was added to the CPB circuit before reoxygenation (Fig. 4).

Discussion

This study in immature piglets documents that abrupt reoxygenation after a brief period of hypoxemia produces myocardial dysfunction associated with signs of lipid peroxidation and depletion of endogenous antioxidants that can be avoided by adding CoQ₁₀ to the CPB circuit before reoxygenation. The optimal CoQ₁₀ dose was determined by in vitro studies. These results were then transferred to the in vivo model of hypoxemia on CPB to avoid the hemodynamic deterioration and acidosis that characterize the ventilator hypoxemia model and to

provide the first evidence that CoQ₁₀ limits cardiac reoxygenation injury in immature subjects.

The *in vivo* model of hypoxemia on CPB provides a reproducible experimental setting that avoids hemodynamic deterioration during hypoxemia and ensures optimal systemic and myocardial blood supply. Nevertheless, we acknowledge the limitations of the acute hypoxemic model, because chronic cyanosis results in myocardial metabolic adaptations to maintain normal metabolism¹⁹ that do not occur in the acute preparation. The acute model, however, has some relevance to the clinical situation, because similar biochemical and functional results occur when hypoxemic infants undergo reoxygenation on CPB.^{7, 9}

Ventricular dysfunction after CPB in cyanotic heart disease may develop or persist after successful anatomic correction of congenital cardiac defects.²⁰⁻²² In the past this has been attributed to several pre-operative and peri-operative factors, including myocardial damage secondary to cyanosis, abnormal cardiac loading, and intraoperative ischemic injury (i.e., global ischemia).^{20, 21} Our findings suggest that reoxygenation injury is an additional causative factor, because signs of lipid peroxidation followed reoxygenation and depletion of endogenous antioxidants was observed after CPB despite the absence of surgically imposed ischemia. These findings suggest that reoxygenated myocardium may become more susceptible to subsequent ischemic insults during cardiac repair. This speculation is supported by the experimental studies in the chronically cyanotic newborn lamb²³ and clinical observations showing impaired myocardial protection in cyanotic versus noncyanotic patients.⁸

Exogenous CoQ₁₀ has been used extensively in studies of regional^{24, 25} and global ischemia^{26, 27} and heart preservation in adult hearts.¹⁴ Ohhara and colleagues²⁶ reported that pretreatment with CoQ₁₀ preserved myocardial stores of adenosine triphosphate and total adenine nucleotides during ischemia in the isolated rat heart preparation, which implies that CoQ₁₀ could maintain mitochondrial oxidative phosphorylation and adenosine triphosphate-generating capacity during ischemia. In this study, we used soy lecithin-solubilized CoQ₁₀, which was developed to facilitate rapid distribution into the myocardium, and the dosage of CoQ₁₀ (45 mg/kg) was derived from previous experimental studies^{14, 24, 25} using intravenous administration of this drug. The CoQ₁₀ dose added to the CPB circuit was determined by considering the dilutional effects of the

Fig. 4. Antioxidant reserve capacity. Vulnerability of hypoxemic and reoxygenated myocardium to subsequent oxidant stress determined by exposing tissue to 0 to 4 mmol/L *t*-butylhydroperoxide for 30 minutes at 37°C. MDA, Malondialdehyde (see text for description). Control: piglets without CPB, no treatment; piglets undergoing hypoxemia/reoxygenation without CoQ₁₀, and CoQ₁₀; piglets treated with CoQ₁₀. **p* < 0.05 vs control and CoQ₁₀; *t*-BHP, *t*-butylhydroperoxide.

added volume for priming CPB circuit and conformed to optimal dose achieved by *in vitro* analysis (Fig. 1). Takada and colleagues²⁸ reported that 3.3% of a radioactive dose of soy lecithin-[¹⁴C] CoQ₁₀ entered the respiratory chain of the myocardial mitochondria within 30 minutes after injection, but we did not make direct mitochondrial measurement to confirm this. Nakamura and coworkers²⁹ reported that exogenous [¹⁴C] CoQ₁₀ accumulates in the inner membrane and matrix of mitochondria at 72 hours after intravenous administration. In addition, Suzuki and colleagues³⁰ observed that exogenous CoQ₁₀ entered the mitochondrial plasma membrane and cytosolic fraction of cultured myocytes without affecting the content of endogenous CoQ₁₀. Therefore it is conceivable that exogenous CoQ₁₀ in the present experimental setting may act directly on the plasma and mitochondrial membrane to protect against myocardial injury without accumulation in the mitochondria.

The antioxidant properties of CoQ₁₀, like tocopherol, are documented by *in vitro* studies of subcellular systems, including phospholipid vesicles,³¹ submitochondrial particles, mitochondria,³² and microsomes.³³ Furthermore, the antioxidant properties of CoQ₁₀ have been demonstrated recently after ischemic stress.^{13, 14} The present studies of hypoxemic stress indicate that CoQ₁₀ avoids the depression of myocardial con-

tractility, rise of conjugated diene levels, and reduction of antioxidant reserve that otherwise follows reoxygenation. Hydroxy conjugated dienes are free fatty acid oxidation products that are possible markers of lipid peroxidation,³⁴ and studies in isolated heart models document the association of lipid peroxidation and myocardial dysfunction^{35, 36} after reoxygenation. Our findings suggest strongly the salutary effects of adding CoQ₁₀ to the CPB circuit are related to its antioxidant effects.

In our in vitro study, incubation with CoQ₁₀ resulted in dose-dependent suppression of lipid peroxidation in myocardial homogenates exposed to *t*-butylhydroperoxide. Lipid peroxidation in this in vitro preparation is due to hydroxyl radicals and hydrogen peroxide-generating system, because both *N*-(2-mercaptopropionyl)-glycine and catalase produce similar inhibition of malondialdehyde production, whereas superoxide dismutase is ineffective.³⁷ We suspect that CoQ₁₀ reacted with lipid free radicals or lipid peroxide radicals and limited the propagation reaction of lipid peroxidation. The concentrations of CoQ₁₀ showing antioxidant properties in the present in vitro study (100 to 400 μ mol/L) are consistent with those of Beyer,³³ who used rat liver microsomes, and could be achieved in the plasma at 30 minutes after intravenous administration of 15 mg/kg of this agent.²⁵ Unfortunately, neither parenteral CoQ₁₀ nor other antioxidants, such as *N*-(2-mercaptopropionyl)-glycine and catalase, can be used clinically, because they are not yet approved for intravenous administration. However, CoQ₁₀ is approved for oral administration in children and adult, and blood levels similar to those achieved by intravenous injection have been reported after 10 days of oral administration.²⁵

We conclude that abrupt reoxygenation after brief periods of hypoxemia on CPB produces myocardial dysfunction associated with signs of lipid peroxidation and depletion of endogenous antioxidants and that this oxygen-mediated reoxygenation injury can be limited by adding CoQ₁₀ to the CPB circuit 15 minutes before reoxygenation. These findings imply that CoQ₁₀ can be used to surgical advantage in cyanotic patients if our data are confirmed by others, because therapeutic blood levels can be achieved by preoperative oral administration of this approved drug.

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REFERENCES

1. Touati GD, Vouhe PR, Amodeo A. Primary repair of tetralogy of Fallot in infancy. *J THORAC CARDIOVASC SURG* 1990;99:396-403.
2. Kirklin JK, Blackstone EH, Kirklin JW, McKay R, Pacifico AD, Barger LMJ. Intracardiac surgery in infants under age 3 months: incremental risk factors for hospital mortality. *Am J Cardiol* 1981;48:500-6.
3. Teoh KH, Mickle DAG, Weisel RD, et al. Effect of oxygen tension and cardiovascular operations on the myocardial antioxidant enzyme activities in patients with tetralogy of Fallot and aorta-coronary bypass. *J THORAC CARDIOVASC SURG* 1992;104:159-64.
4. Cavarocchi NC, England MD, Schaff HV, Russo P, Orszulak T, Schnell WAJ, O'Brien JF, Pluth JR. Oxygen free radical generation during cardiopulmonary bypass: correlation with complement activation. *Circulation* 1986;74:III130-III3.
5. Buckberg GD. Studies of hypoxemic/reoxygenation injury without aortic clamping. I. Linkage between cardiac function and oxidant damage: an overview. *J THORAC CARDIOVASC SURG* 1995;110:1166-70.
6. Ihnken K, Morita K, Buckberg GD, Matheis G, Sherman MP, Allen BS, Young HH. Studies of hypoxemia/reoxygenation injury without aortic clamping. II. Evidence for reoxygenation damage. *J THORAC CARDIOVASC SURG* 1995;110:1171-81.
7. Del Nido PJ, Mickle DAG, Wilson GJ, Benson LN, Coles JG, Trusler GA, Williams WG. Evidence of myocardial free radical injury during elective repair of tetralogy of Fallot. *Circulation* 1987;76(suppl V):174-9.
8. Del Nido PJ, Mickle DAG, Wilson GJ, et al. Inadequate myocardial protection with cold cardioplegic arrest during repair of tetralogy of Fallot. *J THORAC CARDIOVASC SURG* 1988;95:223-9.
9. Hirschl RB, Heiss KF, Bartlett RH. Severe myocardial dysfunction during extracorporeal membrane oxygenation. *J Pediatr Surg* 1992;27:48-53.
10. Matheis G, Sherman MP, Buckberg GD, Haybron DM, Young HH, Ignarro LJ. Role of L-arginine-nitric oxide pathway in myocardial reoxygenation injury. *Am J Physiol* 1992;262:H616-H20.
11. DePierre JW, Ernster L. Enzyme topology of intracellular membranes. *Annu Rev Biochem* 1977;46:201-62.
12. Beyer RE. The participation of coenzyme Q in free radical production and antioxidation. *Free Radic Biol Med* 1990;8:545-65.
13. Marubayashi S, Dohi K, Yamada K, Kawasaki T. Changes in the levels of endogenous coenzyme Q homologs, alpha-tocopherol, and glutathione in rat liver after hepatic ischemia and reperfusion, and the effect of pretreatment with coenzyme Q10. *Biochem Biophys Acta* 1984;797:1-9.
14. Matsushima T, Sueda T, Matsuura Y, Kawasaki T. Protection by coenzyme Q10 of canine myocardial

- reperfusion injury after preservation. *J THORAC CARDIOVASC SURG* 1992;103:945-51.
15. Little WC, Cheng C, Mumma M, Igarashi Y, Vinten-Johansen J, Johnston WE. Comparison of measures of left ventricular contractile performance derived from pressure-volume loops in conscious dogs. *Circulation* 1989;80:1378-87.
16. Sagawa K, Maughan L, Suga H, Sunagawa K. Cardiac contraction and the pressure-volume relationship. New York: Oxford University Press, 1988.
17. Lesnefsky EJ, Fennessey PM, Van Benthuyzen KM, McMurtry IF, Travis VL, Horwitz LD. Superoxide dismutase decreases early reperfusion release of conjugated dienes following regional canine ischemia. *Basic Res Cardiol* 1989;84:191-6.
18. Godin DV, Ko KM, Garnett ME. Altered antioxidant status in the ischemic/reperfused rabbit myocardium: effects of allopurinol. *Can J Cardiol* 1989;5:365-71.
19. Rudolph W. Myocardial metabolism in cyanotic congenital heart disease. *Cardiology* 1971;56:209-15.
20. Borow KM, Keane JF, Castaneda AR, Freed MD. Systemic ventricular function in patients with tetralogy of Fallot, ventricular septal defect and transposition of the great arteries repaired during infancy. *Circulation* 1981;64:878.
21. Jarmakani JMM, Graham TPJ, Canent RV, Jewett PH. Left heart function in children with tetralogy of Fallot before and after palliative or corrective surgery. *Circulation* 1972;XLVI:478-88.
22. Arensman FW, Radley-Smith R, Yacoub MH, Lange P, Bernhard A, Sievers HN, Heintzen P. Catheter evaluation of left ventricular shape and function 1 or more years after anatomic correction of transposition of the great arteries. *Am J Cardiol* 1983;52:1079-83.
23. Fujiwara T, Kurtts T, Anderson W, Heinle J, Mayer JEJ. Myocardial protection in cyanotic neonatal lambs. *J THORAC CARDIOVASC SURG* 1988;96:700-10.
24. Okamoto F, Allen BS, Buckberg GD, Leaf J, Bugyi HI. Studies of controlled reperfusion after ischemia: reperfusate composition: X. Supplemental role of intravenous and intracoronary CoQ10 in avoiding reperfusion damage. *J THORAC CARDIOVASC SURG* 1986;92:573-82.
25. Imai S, Tamatsu H, Ushijima T, et al. Effect of CoQ10 on myocardial energy metabolism in ischemia. In: Folkers K, Yamamura Y, eds. *Biomedical and clinical aspects of coenzyme Q*. 4th ed. Amsterdam: Elsevier, 1984;315-23.
26. Ohhara H, Kanaide H, Yoshimura R, Okada M, Nakamura M. A protective effect of coenzyme Q10 on ischemia and reperfusion of the isolated perfused rat heart. *J Mol Cell Cardiol* 1981;13:65-74.
27. Mori F, Mohri H. Effects of coenzyme Q10 added to a potassium cardioplegic solution for myocardial protection during ischemic cardiac arrest. *Ann Thorac Surg* 1985;39:30-5.
28. Takada M, Yuzuriha T, Katayama K, Yamato C, Koyama N. Targeting coenzyme Q10 solubilized with soy lecithin to heart of guinea pigs. *J Nutr Sci Vitaminol* 1985;31:115-20.
29. Nakamura T, Sanma H, Himeno M, Kato K. Transfer of exogenous coenzyme Q10 to the inner membrane of heart mitochondria in rats. In: Yamamura Y, Folker K, Ito Y, eds. *Biomedical and clinical aspects of coenzyme Q*. 2nd ed. Amsterdam: Elsevier, 1980: 3-14.
30. Suzuki N, Nakamura T, Ishida H, Hosono K. Protective effect of coenzyme Q10 against hypoxic cellular damage. *Chem Pharm Bull* 1985;33:2896-903.
31. Landi L, Fiorentini D, Cabrini L, Stefanelli C, Sechi AM. Effects of free radicals produced by sonolysis on ubiquinone-containing vesicles. *Biochem Biophys Acta* 1989;984:21-5.
32. Mellors A, Tappel AL. The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *J Biol Chem* 1966;241:4353-6.
33. Beyer RE. Inhibition by coenzyme Q of ethanol- and carbon tetrachloride-stimulated lipid peroxidation in vivo and catalyzed by microsomal and mitochondrial systems. *Free Radic Biol Med* 1988;5:297-303.
34. Romaschin AD, Rebeyka I, Wilson GJ, Mickle DAG. Conjugated dienes in ischemic and reperfused myocardium: an in vivo chemical signature of oxygen free radical mediated injury. *J Mol Cell Cardiol* 1987;19: 289-302.
35. Guarnieri C, Flamigni F, Caldarera CM. Role of oxygen in the cellular damage induced by reoxygenation of hypoxic heart. *J Mol Cell Cardiol* 1980;12: 797-808.
36. Dhaliwal H, Kirshenbaum LA, Randhawa AK, Singal PK. Correlation between antioxidant changes during hypoxia and recovery on reoxygenation. *Am J Physiol* 1991;261:H632-H8.
37. Ihnken K, Morita K, Buckberg GD, Sherman MP, Young HH. Studies of hypoxemia/reoxygenation injury without aortic clamping. VI. Counteraction of oxidant damage by exogenous antioxidants: MPG and catalase. *J THORAC CARDIOVASC SURG* 1995;110:1212-20.