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Dietary coenzyme Q₁₀ supplement renders swine hearts resistant to ischemia-reperfusion injury

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Maulik, Nilanjana, Tetsuya Yoshida, Richard M. Engelman, Debasis Bagchi, Hajime Otani, and Dipak K. Das. Dietary coenzyme Q₁₀ supplement renders swine hearts resistant to ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 278: H1084–H1090, 2000.—To examine whether nutritional supplementation of coenzyme Q₁₀ (CoQ₁₀) can reduce myocardial ischemia-reperfusion injury, a group of swine was fed a regular diet supplemented with CoQ₁₀ (5 mg·kg⁻¹·day⁻¹) for 30 days. Another group of pigs that were fed a regular diet supplemented with placebo served as a control. After 30 days, isolated in situ pig hearts were prepared and hearts were perfused with a cardiopulmonary pump system. Each heart was subjected to 15 min of regional ischemia by snaring of the left anterior descending coronary artery, followed by 60 min of hypothermic cardioplegic global ischemia and 120 min of reperfusion. After the experiments were completed, myocardial infarct size was measured by triphenyltrazolium chloride staining methods. Postischemic left ventricular contractile function was better recovered in the CoQ₁₀ group than in the control group of pigs. CoQ₁₀-fed pigs revealed less myocardial infarction and less creatine kinase release from the coronary effluent compared with control pigs. The experimental group also demonstrated a smaller amount of malonaldehyde in the coronary effluent and a higher content of the endogenous antioxidants ascorbate and thiol. Significant induction of the expression of ubiquitin mRNA was also found in the hearts of the CoQ₁₀-fed group. The results of this study demonstrate that nutritional supplementation of CoQ₁₀ renders the hearts resistant to ischemia-reperfusion injury, probably by reducing the oxidative stress.

ubiquitin; ubiquinone; oxygen free radicals; oxidative stress

A LIPID SOLUBLE BENZOQUINONE, coenzyme Q₁₀ (CoQ₁₀), is an essential component for electron transport in oxidative phosphorylation of mitochondria. Also called ubiquinone, its principal function is to act as an electron carrier between the NADH and succinate dehydrogenases and the cytochrome system (35). During mitochondrial electron transport, ubiquinone also occurs as semiquinone and ubiquinol, the fully reduced form of ubiquinone. Semiquinone has a role in the generation

of superoxide anions during mitochondrial respiration (22), whereas ubiquinol functions as an intracellular antioxidant, presumably by preventing both the initiation and propagation of lipid peroxidation (13).

CoQ₁₀ appears to be involved in the coordinated regulation between oxidative stress and antioxidant capacity of heart tissue. When the heart is subjected to oxidative stress in various pathogenic conditions (9, 23), the amount of CoQ₁₀ is decreased, which triggers a signal for increased CoQ₁₀ synthesis. It has been reported that in patients with cardiac disease such as chronic heart failure, the myocardium becomes deficient in CoQ₁₀ and CoQ₁₀ reductase (35). CoQ₁₀ level is also reduced in other cardiovascular diseases such as cardiomyopathy (28). CoQ₁₀ can protect human low-density lipoprotein (LDL) from lipid peroxidation, suggesting its role in atherosclerosis (33). Several reports exist in the literature indicating cardioprotective effects of CoQ₁₀ against ischemia-reperfusion injury (15, 28, 34). However, none of these studies attempted to evaluate the mechanism(s) of CoQ₁₀-mediated cardioprotection, and none demonstrated whether postischemic improvement of myocardial function was caused by the improvement of an endogenous defense system.

In the present study, the mechanism of action of CoQ₁₀ was examined by feeding pigs CoQ₁₀ for 1 mo in an attempt to increase the CoQ₁₀ content of the heart. The results of this study demonstrated that CoQ₁₀-fed pigs were resistant to myocardial ischemia-reperfusion injury. The hearts of CoQ₁₀-fed animals had higher levels of CoQ₁₀, higher levels of the intracellular antioxidants ascorbate and thiol, and an increased amount of ubiquitin gene expression, which may be attributed to its ability for being resistant to ischemic injury.

MATERIALS AND METHODS

Measurement of myocardial CoQ₁₀. For CoQ₁₀ assay, the left ventricular (LV) biopsies from the ischemic region were quickly frozen in liquid nitrogen. Frozen tissues were stored at -70°C for subsequent determination of CoQ₁₀. At a later date, the tissues were homogenized and treated with 2% ferric chloride to convert the reduced form of ubiquinone into the oxidized form, which was extracted with *n*-hexane. The solvent was evaporated at 30°C under nitrogen. The residue was dissolved in isopropyl alcohol and analyzed by HPLC (11). CoQ₁₀ was eluted with methanol-ethanol (1:1 vol/vol) by

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being passed through a C18 column. Ultraviolet absorbance of the elute was monitored at 275 nm.

Treatment with CoQ₁₀. Male Yorkshire pigs weighing 18–25 kg were used in this study. 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 Institute of Laboratory Animal Resources and published by the National Research Council (Revised 1996). Twelve pigs were fed coenzyme Q₁₀ (5 mg·kg⁻¹·day⁻¹; UAS Laboratories, Minnetonka, MN) supplemented with regular laboratory diet for 30 days. Twelve age-matched pigs that were fed regular diets plus placebo served as controls.

Experimental preparation. At the end of 30 days, each pig was tranquilized with ketamine (20 mg/kg body wt) and anesthetized with pentobarbital sodium (25 mg/kg body wt). Endotracheal intubation was performed, and ventilation was maintained by a volume respirator with room air. A median sternotomy was performed, and the azygos vein was ligated (25). The pericardium was incised and suspended in a pericardial cradle. After heparinization with heparin sodium (300 U/kg body wt), an arterial cannula was placed in the ascending aorta through the right carotid artery and a venous cannula was placed in the right atrium. A cannula was also placed in the left atrium through the appendage to control preload for measurements of ventricular function. Sonometric dimension crystals (diameter 6 mm) made of 3-MHz piezoelectric crystals (Triton Technologies, San Diego, CA) were placed at the endocardial surface across the anteroposterior minor axis, septal-free wall minor axis, and base-apex major axis of the left ventricle. The anteroposterior crystals were placed adjacent to the anterior and posterior descending coronary arteries. The septal-free crystals were located one-half the distance from the apex to the base. The base crystal was placed into the left ventricle adjacent to the origin of the left circumflex coronary artery, and the apex crystal was placed into the LV apex. Cardiopulmonary bypass with a membrane oxygenator was initiated, and blood was collected from the pig in a reservoir. The heart was then isolated in its own perfusion circuit by completely cross-clamping the ascending aorta just distal to the right brachiocephalic artery distal to the arterial inflow and ligating both superior and inferior vena cavae. The main pulmonary trunk was drained. This achieves complete cessation of systemic circulation while coronary perfusion is maintained.

On stable bypass, the left anterior descending coronary artery (LAD) was then snared just distal to the first diagonal branch. After 15 min of normothermic regional ischemia, the aorta was clamped and the ligature removed from the LAD. Normothermic cardioplegic arrest was initiated. Initial high-K⁺ blood cardioplegic solution (K⁺ 20–24 meq/l, 20 ml/kg) was administered through the carotid cannula into the coronary circulation at a flow rate that insured a perfusion pressure of 50–75 mmHg. This high-K⁺ blood cardioplegic solution arrested each heart promptly. Additional low-K⁺ blood cardioplegic solution (K⁺ 8–12 meq/l, 10 ml/kg) was administered every 15 min (at 15, 30, and 45 min) for a total of 60 min of arrest.

After 60 min of cardioplegic arrest, the heart was reperfused on cardiopulmonary bypass. The snare of the LAD was released just before reperfusion. Normothermia was maintained with a heat exchanger, and reperfusion was continued for a total of 180 min. Coronary perfusion was maintained at ≥ 75 mmHg. Defibrillation was applied when the heart suffered ventricular fibrillation during reperfusion. The heart was atrially paced at 120 beats/min. No cardiotoxic or antiar-

rhythmic drugs were administered during the experiment. Blood samples were taken through the pulmonary arterial cannula (coronary sinus blood) at 0 min (baseline) and during the reperfusion for malonaldehyde (MDA) and creatine kinase (CK) measurements. Before regional ischemia and at the end of experiment, LV biopsies were taken for biochemical determination.

Functional data were obtained by adding 60 ml of saline through the left atrial cannula to raise the LV end-diastolic pressure, with subsequent withdrawal of the saline while maximal developed pressure and end-diastolic pressure were measured before ischemia (control) and during reperfusion. The first derivative of LV pressure (LV dP/dt) was calculated as a polynomial approximation from the digital LV pressure signal. LV dP/dt_{max} was obtained when the point of the LV pressure-volume loop showed peak LV developed pressure (LVDP).

Estimation of CK release from heart. CK was quantified from 0.5 μ l of plasma by the enzymatic assay method using a CK assay kit (Sigma Diagnostics, St. Louis, MO). The absorbance was read at 340 nm using a Beckman DU-8 spectrophotometer. The enzyme activity was expressed in units per milliliter.

Assessment of myocardial infarction. After 2 h of reperfusion, triphenyltetrazolium chloride (TTC; 10 ml, 1% solution) in phosphate buffer preheated to 37°C was injected directly into the coronary arteries through the ascending aorta at 70–75 mmHg after the distal ascending aorta was clamped. We did not attempt to measure the area of risk because it is well known that pig hearts have little collateral coronary circulation. However, the area of risk was identical in the two groups when the LAD was clamped just distal to the first diagonal branch. After a 10-min incubation, noninfarcted myocardium was stained red and infarct area was unstained. The heart was stored at –20°C, and frozen slices were analyzed with sections from apex to base. The infarct area was calculated as a percentage of the total LV myocardium.

Thin sections were placed between plates and scanned using NIH Image processing software. Each digitized image was subjected to background subtraction and contrast enhancement. The total left ventricle and the area of infarction were traced and the respective areas calculated in terms of pixels. The infarct volume was calculated, and the sum of all slices was used to compute a ratio of percent infarct to total LV area.

Measurement of MDA formation. MDA was assayed as described previously to estimate the lipid peroxidation (5). Plasma (1.5 ml) was mixed with an equal volume of 20% TCA and 5.3 mM sodium metabisulfite. Protein was precipitated on ice for 10 min. Two milliliters of supernatant were derivatized with 2,4-dinitrophenylhydrazine and extracted with pentane. MDA formation was then measured using HPLC (5).

Determination of antioxidant reserve. The antioxidant reserve (12, 33) of each heart was measured on the basis of its ability to reduce the phenoxyl radical of a hindered phenol, 4'-dimethyl-epipodophyllotoxin-9-(4,6-*O*-ethylidene- β -D-glucopyranoside) (VP-16), generated as an intermediate in the oxidation of phenoxyl radical by tyrosinase (12). Reduction of the generated VP-16 phenoxyl radical by endogenous antioxidants results in a delay (lag period) in the appearance of the characteristic electron spin resonance (ESR) signal of the VP-16 radical.

Hearts were homogenized in ice-cold 0.1 M phosphate buffer (iron-free), pH 7.4, using a Polytron homogenizer. ESR measurements were performed with an ESR spectrometer using a sample volume of 0.1 ml containing 500 μ M VP-16, 3

U/μl tyrosinase, 25 μl of heart homogenate (20 mg protein/ml), and 100 μM deferoxamine. The lag period was determined as the time interval elapsed from the beginning of the measurement to the appearance of VP-16 phenoxyl radical signal, as described previously (12, 27).

Assessment of ubiquinone gene expression. LV biopsies obtained for ubiquitin mRNA determination were immediately frozen in liquid nitrogen and stored at -70°C for subsequent mRNA determination. Total RNA was extracted from the heart by the acid-guanidinium-thiocyanate-phenol-chloroform method as previously described by Maulik et al. (26). For Northern blot analysis, total RNA was electrophoresed in 1% agarose-formaldehyde-formamide gel and transferred to Gene Screen Plus. After prehybridization, membranes were hybridized with a 780-bp (*Hind* II/*Bam* H I) cDNA insert encoding porcine ubiquitin (32). Each hybridization was repeated at least three times using different membranes. After each hybridization, ubiquitin cDNA was removed and rehybridized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe, the results of which served as loading controls. The autoradiographs were quantitatively evaluated by a computerized beta scanner. The results of densitometric scanning were normalized relative to the signal obtained with GAPDH cDNA probe.

Statistical analysis. The results are expressed as means ± SE. An ANOVA was carried out first to compare variance between groups. Differences between groups were analyzed by a two-tailed Student's *t*-test. A *P* value <0.05 was considered statistically significant.

RESULTS

Effects of CoQ₁₀ feeding on myocardial CoQ₁₀ content. To ensure the bioavailability of CoQ₁₀ in the heart, the amount of CoQ₁₀ was estimated in the hearts of both CoQ₁₀-fed and non-CoQ₁₀-fed groups. Hearts of the CoQ₁₀-fed group showed significantly higher CoQ₁₀ content (28 ± 0.5 μg/g heart) compared with controls (21 ± 0.7 μg/g heart) (Fig. 1). After ischemia, control hearts demonstrated a lower CoQ₁₀ content (19.2 ± 0.9 μg/g heart) than the baseline values. In contrast, the amount of CoQ₁₀ remained unaltered in the CoQ₁₀-fed hearts (27.3 ± 0.8 μg/g heart). Two hours of reperfusion

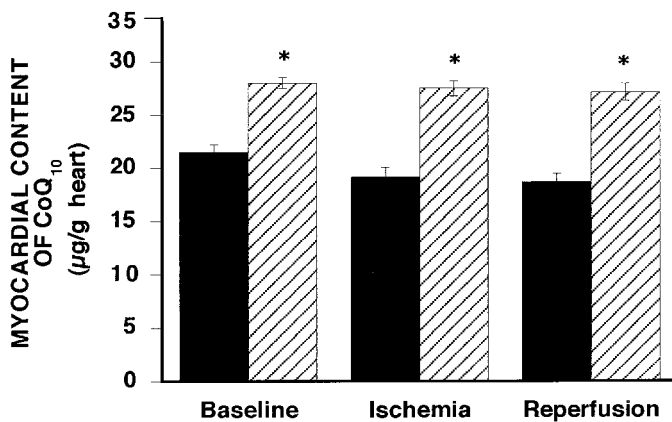


Fig. 1. Effects of coenzyme Q₁₀ (CoQ₁₀) feeding on CoQ₁₀ content of heart. Myocardial content of CoQ₁₀ was estimated in control (filled bars) and CoQ₁₀-fed pigs (hatched bars) as described in MATERIALS AND METHODS. CoQ₁₀ content was measured in left ventricular (LV) biopsies at baseline, after ischemia, and after reperfusion. Results are expressed as means ± SE of 6 animals/group. **P* < 0.05 compared with control.

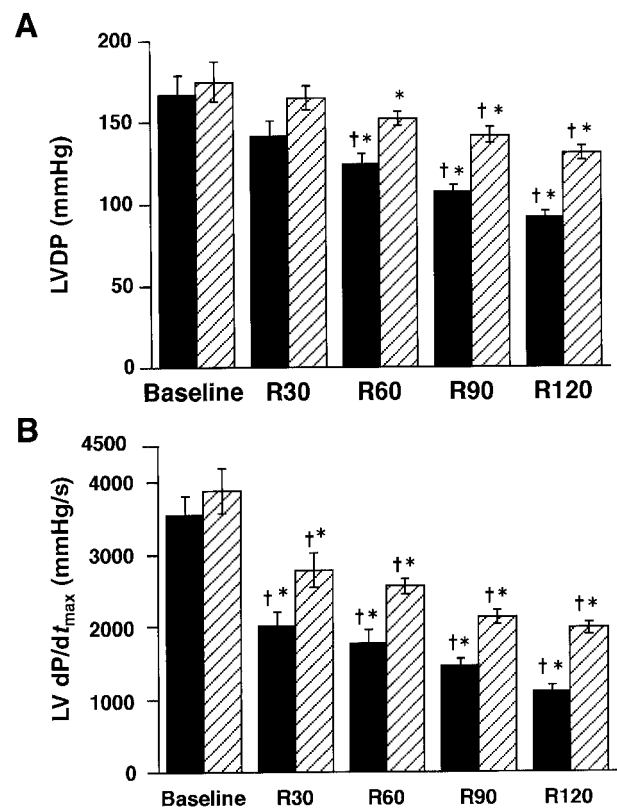


Fig. 2. Effects of CoQ₁₀ feeding on LV functions. LV developed pressure (LVDP) (A) and maximal first derivative of LV pressure (LV dP/dt_{max}) (B) were measured in control (filled bars) and CoQ₁₀-fed pigs (hatched bars) as described in MATERIALS AND METHODS. Hearts of CoQ₁₀-fed pigs demonstrated significantly better LV function compared with hearts of corresponding control pigs at 60, 90, and 120 min of reperfusion (R). Results are expressed as means ± SE of 6 animals/group. **P* < 0.05 compared with control. †*P* < 0.05 compared with baseline.

did not alter these values significantly in either group of hearts.

LV function. Hemodynamic parameters with a load of 60 ml of saline in control and treatment groups are shown in Fig. 2. The CoQ₁₀-fed group consistently demonstrated higher recovery of postischemic LV systolic function. For example, LVDP was lowered after 30 min of reperfusion in the non-CoQ₁₀-fed group to 142 ± 9.5 mmHg compared with 165 ± 7.2 mmHg in the CoQ₁₀-fed group. The same trend persisted up to 120 min of reperfusion, when LVDP in the control group was 92 ± 3.9 mmHg compared with 131 ± 4.2 mmHg in the CoQ₁₀-fed group. LV dP/dt_{max} followed a similar pattern. Thus, at the end of reperfusion, these values were 1,110 ± 98 mmHg/s for the control group versus 1,976 ± 85 mmHg/s for the CoQ₁₀-fed group.

Tissue injury. CK release from the heart truly reflects cellular injury and tissue necrosis. As shown in Fig. 3, CK release increased steadily and progressively in both groups. However, the amount of CK release in the hearts of the CoQ₁₀-fed group was consistently lower than that in hearts of the non-CoQ₁₀-fed group. For example, after 30 min of reperfusion, the amount of CK release from the control hearts was 112 ± 5.8 IU/l compared with 72 ± 5.6 IU/l from the CoQ₁₀-fed hearts.

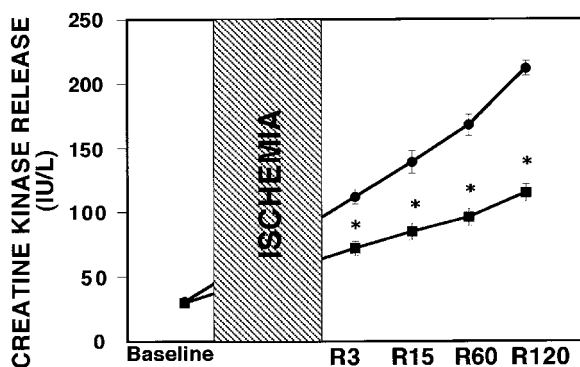


Fig. 3. Effects of CoQ₁₀ feeding on creatine kinase (CK) release from heart. CK release in CoQ₁₀-fed pigs (■) is significantly lower than that in control pigs (●). Results are expressed as means \pm SE of 6 animals/group. * $P < 0.05$ compared with control.

At the end of 2 h of reperfusion, CK release from the control heart was 212 ± 5.9 IU/l compared with only 115 ± 6.9 IU/l for the CoQ₁₀-fed pig hearts.

Infarct size for each heart was expressed as (sum of infarct area of each slice/sum of total LV area of each slice) \times 100. The mean value of infarct size in the CoQ₁₀-fed group was significantly smaller than that in the non-CoQ₁₀-fed group (Fig. 4). For example, infarct size for the CoQ₁₀-fed group was only $19.5 \pm 2.0\%$ compared with $35.0 \pm 2.8\%$ for the non-CoQ₁₀-fed group of hearts. Our results thus indicate that CoQ₁₀ feeding resulted in reduced myocardial tissue damage as documented by less CK release and smaller myocardial infarction.

Reduction of oxidative stress by CoQ₁₀. The amount of oxidative stress in the heart was determined by examining the free radical-lipids interaction product, lipid peroxidation, by monitoring the amount of MDA content in the coronary effluent (3, 5). An increased amount of MDA was found in all groups during the early reperfusion, as expected. However, as shown in Fig. 5, the amount of MDA was significantly lower in the CoQ₁₀-fed group than in the non-CoQ₁₀-fed group at each time point tested, suggesting that CoQ₁₀ resulted

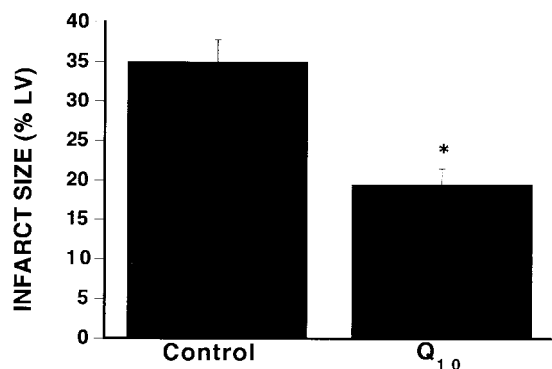


Fig. 4. Effects of CoQ₁₀ feeding on myocardial infarct size. Infarct size (expressed as %LV) was estimated in control and CoQ₁₀-fed pigs (Q₁₀) at end of each experiment as described in MATERIALS AND METHODS. Hearts of CoQ₁₀-fed pigs showed a significantly lower amount of infarct size than did hearts of control pigs. Results are expressed as means \pm SE of 6 animals/group. * $P < 0.05$ compared with control.

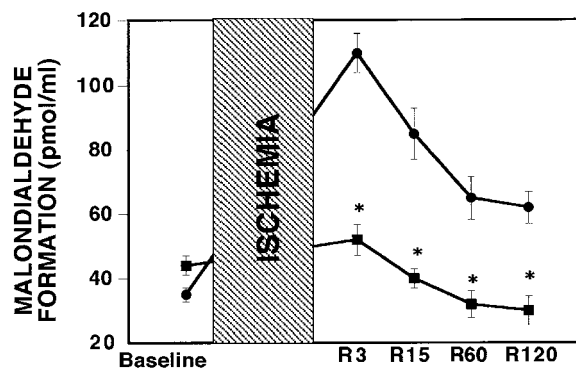


Fig. 5. Effects of CoQ₁₀ feeding on malondialdehyde (MDA) content of heart. MDA content was measured as described in MATERIALS AND METHODS. Hearts of CoQ₁₀-fed pigs (■) showed a lower amount of MDA formation compared with hearts of control pigs (●), indicating a smaller degree of oxidative stress in hearts of CoQ₁₀-fed pigs. Results are expressed as means \pm SE of 6 animals/group. * $P < 0.05$ compared with control.

in the reduction of oxidative stress that is developed during ischemia and reperfusion. At 3 min of reperfusion following ischemia, the MDA concentration was 110 ± 6 pmol/ml for the control group versus 52 ± 4.8 pmol/ml for CoQ₁₀-fed pig hearts. Even after 2 h of reperfusion, the amount of MDA released from the control hearts was 62 ± 5 pmol/ml compared with 30 ± 4.5 pmol/ml from the CoQ₁₀-fed hearts.

Effects of CoQ₁₀ on antioxidant reserve of the heart. The VP-16 phenoxyl radical formed as an intermediate in the VP-16 oxidation by tyrosinase was detected. In the presence of heart homogenates, the characteristic ESR signal of the VP-16 phenoxyl radical could be observed only after a lag period. Heart homogenates from the non-CoQ₁₀-fed animals showed a significantly lower amount of lag period for the appearance of the tyrosinase-induced VP-16 phenoxyl radical ESR signal (Fig. 6). A significantly greater amount of lag period was found for CoQ₁₀-fed heart homogenates, suggesting greater antioxidant reserve in the hearts of CoQ₁₀-fed pigs.

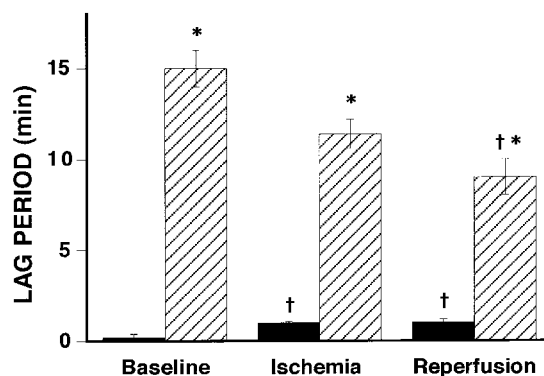


Fig. 6. Effects of CoQ₁₀ feeding on antioxidant reserve of heart. Estimation of lag period of VP-16 phenoxyl radical generation by tyrosinase-catalyzed VP-16 oxidation in presence of heart homogenate from control (filled bars) and CoQ₁₀-fed pig hearts (hatched bars) is shown at baseline, after ischemia, and after reperfusion. Results are expressed as means \pm SE of 6 animals/group. * $P < 0.05$ compared with control. † $P < 0.05$ compared with baseline.

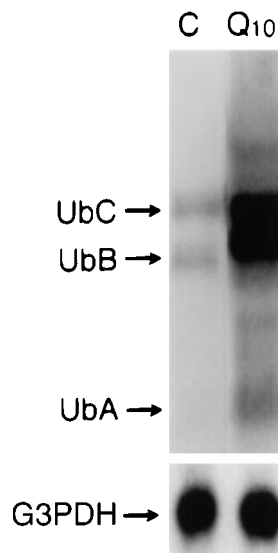


Fig. 7. Effects of CoQ₁₀ feeding on ubiquitin gene expression. Upregulation of ubiquitin gene expression is shown in CoQ₁₀-fed pig hearts. Sizes of 3 different transcripts are 1.3, 2.5, and 3.5 kb for UbA, UbB, and UbC, respectively. C, control; Q₁₀, CoQ₁₀-fed pig; G3PDH, glyceraldehyde-3-phosphate dehydrogenase.

Ubiquitin gene expression. The ubiquitin gene was ubiquitously present in the hearts of all animals. The transcripts UbB and UbC were detected in the hearts of non-CoQ₁₀-fed animals at 2.5 and 3.5 kb, respectively (Fig. 7). Induction of the expression of UbB and UbC was increased significantly in the CoQ₁₀-fed hearts. Additionally, an induction of the expression of UbA was also prominent in the hearts of the CoQ₁₀-fed group only.

DISCUSSION

In the present study, we demonstrated an ~30% increase in the myocardial content of CoQ₁₀ after 30 days of feeding. There are a number of data showing that the tissue level of CoQ₁₀ increases after the administration of exogenous CoQ₁₀ in mammals, including humans (17). Exogenously administered CoQ₁₀ is nonspecifically incorporated into the cell membranes and into various subcellular fractions and organelles, such as mitochondrial membranes and sarcoplasmic reticular membranes (29). Ferrara et al. (15) reported that after 4 wk of dietary supplementation with CoQ₁₀, tissue concentration of CoQ₁₀ was elevated by 22% and oxidative stress was significantly suppressed. In our study, we showed a 30% increase in myocardial CoQ₁₀ content after 1 mo of CoQ₁₀ feeding. After ischemia and reperfusion were completed, the average myocardial CoQ₁₀ content in the treatment group was still higher than that in the control group by 37%. These results corroborated with the findings that total antioxidant reserve in the heart was higher and the amount of oxidative stress was lower in the CoQ₁₀-fed group compared with the non-CoQ₁₀-fed group of hearts. In concert, CoQ₁₀ feeding resulted in higher postischemic ventricular recovery, lower CK release from hearts, and reduced infarct size, suggesting that CoQ₁₀ might be

instrumental in the reduction of myocardial ischemia-reperfusion injury. We measured LVDP and LV dP/dt_{max} after 30, 60, 90, and 120 min of reperfusion. CoQ₁₀-fed animals showed significantly higher levels of LV dP/dt_{max} at each time point, whereas higher levels of LVDP were found only at 60, 90, and 120 min of reperfusion. Although at 30 min of reperfusion the LVDP of CoQ₁₀-fed pigs was higher than that of controls, the difference was not significant.

A significant number of reports exist in the literature to support a role of oxygen free radicals and oxidative stress in ischemia-reperfusion injury (6, 24). The presence of reactive oxygen species has been detected directly using ESR and HPLC techniques and indirectly from the extent of increased lipid peroxidation and DNA and protein damage (3, 4). A variety of antioxidants and antioxidant enzymes such as Mn-superoxide dismutase, catalase, and glutathione-peroxidase were shown to protect cells from oxidative stress and reduce myocardial ischemia-reperfusion injury (8). It was reported that activity of endogenous antioxidants decreased after ischemia and reperfusion (7, 19). In addition to the role of CoQ₁₀ as a component of the mitochondrial respiratory chain, its role as an intracellular antioxidant has gained attention in recent years. However, the precise mechanism of action is not yet well understood. In vitro study demonstrated that the reduced form of CoQ₁₀ protects membrane phospholipid and serum LDL from lipid peroxidation (2). In vivo study reported that CoQ₁₀ reduces myocardial ischemia-reperfusion injury induced by oxidative stress through the suppression of the formation of reactive oxygen species (28, 35). Ferrara et al. (15) showed that long-term CoQ₁₀ supplementation renders protection against oxidative stress induced by ischemia-reperfusion. On the other hand, Hano et al. (18) showed that a beneficial effect is not observed when CoQ₁₀ is added at the onset of reperfusion. Our study showed that long-term administration might enable hearts to increase CoQ₁₀ content, which may be beneficial in protecting the heart from ischemia-reperfusion injury.

In the present study, we used the cardiopulmonary bypass model, which utilizes an isolated in situ heart and mimicks the in vivo ischemia-reperfusion injury. This animal model also simulates human open-heart surgery, because the time course is consistent with human surgery and the heart is subjected to reperfusion with blood. We also adopted hypothermic intermittent blood cardioplegia to protect globally ischemic myocardium. In this model, whole blood was used as a perfusate and the main component of cardioplegia. Whole blood carries leukocytes and oxygen that might be an additional source of free radicals. For example, activated neutrophils during cardiopulmonary bypass generate free radicals (1). Thus the heart was subjected to additional oxidative stress (like in a real situation of open-heart surgery) from blood perfusion in addition to ischemia-reperfusion. Sarcolemmal phospholipids are potential targets for reactive oxygen species, and interaction gives rise to membrane lipid peroxidation. Several aldehydes and ketones such as MDA are the

breakdown products of spontaneous fragmentation (β -cleavage) of peroxides derived from the free radical-polyunsaturated fatty acid interactions. In this study we measured serum MDA, and CoQ₁₀-fed hearts showed a significantly reduced amount of MDA in serum after reperfusion. A significantly higher amount of MDA was found in the control hearts after 3 min of reperfusion, which was quite as expected because free radicals are generated at the onset of reperfusion. CoQ₁₀ blocked this early increase of MDA and maintained lower MDA throughout the reperfusion period. Under normal conditions, free radicals and lipid peroxidation products cannot accumulate in the heart because they are promptly removed by the endogenous protecting system such as antioxidants and antioxidant enzymes that may include endogenous CoQ₁₀.

In this study we adapted a previously described technique for measuring intracellular antioxidants that truly reflects the interactions between the two most important intracellular antioxidants, ascorbate and thiol, in their natural environments (12, 27). To determine tissue antioxidant activity toward phenoxyl radical, we quantitated the ability of heart homogenates from non-CoQ₁₀-fed and CoQ₁₀-fed pigs to reduce an *in vitro* generated phenoxyl radical. The ESR signal with characteristic features of VP-16 phenoxyl radical was apparent before the addition of tissue homogenates that immediately quenched this signal. As described in earlier reports (12, 27), the kinetics of the regeneration of the phenoxyl radical ESR signal following the addition of heart homogenates provides an estimate of two endogenous antioxidants in the hearts: ascorbate and thiol. The results for antioxidant reserve corroborated with the results of MDA formation, demonstrating higher antioxidant reserve and lower oxidative stress in the hearts of CoQ₁₀-fed animals.

In mitochondria, CoQ₁₀ acts as a mobile distributor of reducing equivalents among NADH dehydrogenase, succinate dehydrogenase, and the cytochrome *b-c*₁ segment of the electron transport chain and as a participant in the protonmotive Q cycle responsible for the transfer of protons across the coupling membrane (14). In biological systems, CoQ₁₀ functions as a potent antioxidant, and in its reduced form, ubiquinol, it acts as a free radical scavenger (36). Two different mechanisms of CoQ₁₀ antioxidant function are known to exist: 1) it may act independently as a chain-breaking antioxidant, providing hydrogen atoms to reduce peroxy and/or alkoxy radicals; or 2) a redox interaction may exist between CoQ₁₀ and another lipid-soluble antioxidant, such as α -tocopherol, in its one-electron oxidized form, vitamin E phenoxyl radical (16).

Ubiquitin is a protein of 76 amino acids found in all eukaryotic cells (20). It has been implicated in ATP-dependent proteolysis and exhibits remarkable evolutionary sequence conservation from animal to animal (21). It has been characterized as a heat shock-induced protein in humans, chickens, pigs, and yeast as well as other organisms (10). During the initial periods of heat shock, the protein-ubiquitin conjugation undergoes rapid and pronounced changes, presumably because of

deubiquitination of histone H2A and subsequent accumulation of aberrant proteins. The altered transcription of the ubiquitin gene seems to be essential for restoration of normal activities (30). In the present study, upregulation of ubiquitin gene expression was observed in the hearts of CoQ₁₀-fed pigs. UbA transcript was not found in the hearts of non-CoQ₁₀-fed pigs, whereas UbB and UbC transcripts were identified in these hearts. The levels of UbB and UbC were significantly enhanced, and UbA was induced, in the hearts of pigs fed CoQ₁₀. In recent studies (31, 32), a coordinated expression pattern of ubiquitin and heme oxygenase genes was shown in the same porcine model in which the LAD was occluded for 10 min and reperused for 30 min and after a second occlusion of 10 min followed by 210 min of reperfusion. Given the fact that ubiquitin plays an essential role for degradation of many proteins, the significant induction of ubiquitin gene expression suggests that CoQ₁₀ may be involved in antioxidant adaptation on both degradation of nonessential harmful proteins and induction of new proteins essential for survival in the new environment.

In conclusion, this study demonstrated that nutritional supplementation with CoQ₁₀ provides cardioprotection against ischemia-reperfusion injury. Reduction of oxidative stress in conjunction with increased antioxidant reserve in CoQ₁₀-fed hearts suggests that CoQ₁₀ functions through the improvement of antioxidant reserve of the heart. The present study utilized pig hearts in a cardiopulmonary bypass model. The hearts were subjected to 15 min of LAD occlusion followed by 60 min of hypothermic cardioplegic arrest and 2 h of reperfusion. Infarct size was determined in the hearts subjected to 2 h of reperfusion, whereas the standard practice for TTC infarct size estimation in *in vivo* preparations is a minimum of 3 h of reperfusion.

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