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Coenzyme Q_{10} and exercise training in chronic heart failure

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LV contractility

Aims There is evidence that plasma coenzyme Q_{10} (CoQ_{10}) levels decrease in patients with advanced chronic heart failure (CHF). However, it is not known whether oral CoQ_{10} supplementation may improve cardiocirculatory efficiency and endothelial function in patients with CHF.
Methods and results We studied 23 patients in NYHA class II and III (20 men, three women, mean age 59 ± 9 years) with stable CHF secondary to ischaemic heart disease [ejection fraction 37 ± 7%], using a double-blind, placebo-controlled cross-over design. Patients were assigned to each of the following treatments: oral CoQ_{10} (100 mg tid), CoQ_{10} plus supervised exercise training (ET) (60% of peak VO_2, five times a week), placebo, and placebo plus ET. Each phase lasted 4 weeks. Both peak VO_2 and endothelium-dependent dilation of the brachial artery (EDDBA) improved significantly after CoQ_{10} and after ET as compared with placebo. CoQ_{10} main effect was: peak $VO_2 + 9$%, EDDBA +38%, systolic wall thickening score index (SWTI) −12%; ET produced comparable effects. CoQ_{10} supplementation resulted in a four-fold increase in plasma CoQ_{10} level, whereas the combination with ET further increased it. No side effects were reported with CoQ_{10}.
Conclusions Oral CoQ_{10} improves functional capacity, endothelial function, and LV contractility in CHF without any side effects. The combination of CoQ_{10} and ET resulted in higher plasma CoQ_{10} levels and more pronounced effects on all the abovementioned parameters. However, significant synergistic effect of CoQ_{10} with ET was observed only for peak SWTI suggesting that ET amplifies the already described effect of CoQ_{10} on contractility of dysfunctional myocardium.

Introduction

Coenzyme Q_{10} (CoQ_{10}), first isolated from beef heart mitochondria,[1] is an essential component of the mitochondrial respiratory chain, and also has antioxidant properties.[2] However, its role in chronic heart failure (CHF) is not well defined. The rationale for CoQ_{10} supplementation in CHF lies in at least two factors. One is the well-known role of CoQ_{10} in myocardial bioenergetics, and the second is its antioxidant property. CoQ_{10}, an obligatory component of the mitochondrial electron transport chain, is essential for ATP generation. Its bioenergetic effect is believed to be of fundamental importance, particularly in cells with high metabolic demand such as cardiac myocytes. Previous reports have shown that CoQ_{10} concentration is decreased in myocardial tissue[3] in CHF, and the greater its deficiency, the more severe is the cardiocirculatory impairment.[4] Plasma CoQ_{10} levels are also decreased in severe cardiocirculatory dysfunction[5] as well as in conditions of high oxidative stress, such as diabetes and liver disease.[6] Moreover, it has been hypothesized that an improvement in LV function may be obtained by raising plasma CoQ_{10} availability. However, in advanced heart failure and ischaemic heart disease, oral CoQ_{10} supplementation, at doses close to 100 mg/die, improved left ventricular (LV) systolic function in some studies,[7–14] but not in others.[15,16] A possible explanation may be that CoQ_{10} exerts biological effects when it reaches at least three times the normal plasma range, and that oral doses used in previous studies were too low.[17] Other explanations for these contrasting results may be concomitant medications, such as statins,[18] and/or the choice of insufficiently accurate techniques.

Another important abnormality in CHF is endothelial dysfunction, which contributes to functional impairment. In CHF, endothelial dysfunction may depend either on reduced nitric oxide synthesis, or increased nitric oxide inactivation, or both. Increased oxidative stress has been shown to augment the inactivation of nitric oxide to peroxynitrite; it may not only reduce nitric oxide and prostacyclin availability, but it is also responsible for the progression of atherosclerotic lesions. In recent years, many studies have demonstrated that exercise training (ET) improves the endothelium-dependent relaxation of coronary as well as

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peripheral arteries in CHF, and that this benefit is associated with enhanced functional capacity.[19]

Exercise increases shear stress, which induces endothelial-nitric oxide synthase (e-NOS) expression in coronary arteries, and increased oxidative capacity in skeletal muscle cells.[20] Mainly on the basis of its antioxidant properties, CoQ_{10} might interact with free radicals and play a role in reinforcing the effect of NO-dependent vascular reactivity in coronary as well as peripheral vessels. Coupled with ET, CoQ_{10} may contribute in improving endothelial dysfunction and myocardial function in CHF.

The objective of the present study was to determine whether, in patients with stable moderate CHF, oral CoQ_{10} supplementation given alone or in combination with ET may be more efficient in ameliorating endothelial dysfunction and functional impairment than standard therapy with or without ET. Moreover, we tested the hypothesis that, by raising plasma levels of CoQ_{10} after oral administration of doses three times higher than those used in the past, both LV contractility of dysfunctional myocardium and LV systolic function may be enhanced.

Methods

We studied 23 patients with CHF secondary to ischaemic heart disease (*Table 1*). All patients had documented coronary artery disease (CAD) and performed a coronary angiography in the last 6 months. Inclusion criteria were NYHA II and III CHF clinically stable in the previous 3 months, i.e. no need to change medications in the last 3 months and no hospitalizations for acute heart failure, and ability to exercise. Exclusion criteria were a recent acute coronary syndrome and/or coronary interventions of revascularization (PCI, CABG), renal insufficiency (serum creatinine >2.5 mg/dL), liver abnormalities, uncontrolled hypertension, habitual use of antioxidants (vitamin C, E, A, or CoQ_{10}), orthopaedic, and/or neurological limitations. None of our patients regularly assumed multivitamin/mineral tablets or relevant amount of foods particularly rich in antioxidants.

Study design

The protocol was approved by the Local Ethical Committee. After a run-in period of 1 week, during which patients signed an informed written consent, were visited by a cardiologist and underwent a familiarization cardiopulmonary exercise test. Over a number of 29 recruited patients, six were found ineligible and the 23 eligible ones started a combination of four consecutive treatments (each one lasting 4 weeks), according to a double-blind, placebo-controlled factorial study with a cross-over design: oral CoQ_{10} supplementation (100 mg tid, Q-absorb-100- Jarrow Formulas, Los Angeles, CA, USA), CoQ_{10} plus ET, placebo (tid), and placebo plus ET. Patients were randomized into three groups undergoing three different treatment sequential schemes each one including at least seven patients.

- Group 1: CoQ, CoQ + ET, Placebo, ET
- Group 2: CoQ + ET, Placebo, ET, CoQ
- Group 3: Placebo, ET, CoQ, CoQ + ET

Sample size was determined for $\alpha = 0.05$, $\beta = 0.2$, and taking into account the main endpoints of the study i.e. the effects of CoQ_{10} and ET on VO_2max, ejection fraction (EF), and EDD. For this purpose, we assumed an SD of 1.5/4.5/1.5 and a smallest worthwhile change of 1–1.5/3–4/1–1.5, respectively. The study entry was considered as baseline. At study entry and at the end of each phase, all patients underwent a symptom-limited cardiopulmonary ET, a study of the vasomotor reactivity of the brachial artery, a blood chemistry assessment and a low-dose dobutamine stress echocardiographic (DSE) study. Medications were not changed throughout the study period. Nine out of 23 patients had been on statins, which they discontinued 1 month before starting the present study.

Table 1 Population study

N/Sex (M/F)	23 (20/3)
Age (years)	59 ± 9
Diagnosis, *n* (%)	
Ischaemic cardiomyopathy	23 (100%)
Previous PTCA	5 (21.7%)
Previous CABG	9 (39%)
Coronary risk factors, *n* (%)	
Hypertension	4 (17.4)
Hypercholesterolaemia	8 (34.7)
Diabetes mellitus	6 (26)
Cigarette smoking	5 (22)
LVEF (%)	37 ± 7
NYHA functional class, *n* (II/III)	18/5
Medications, *n* (%)	
Nitrates	5 (21.7)
ACE-I	14 (60.8)
ATA-II	8 (34.7)
BB	12 (61)
Digitalis	6 (26)
Diuretics	7 (30)
ASA	15 (65)
Warfarin	7 (30)

Cardiopulmonary ET

After a familiarization test, a symptom-limited cardiopulmonary exercise test was performed on an electronically braked cycle ergometer using a ramp increase in work rate. Expired gases and volumes were analysed, breath-by-breath, with a metabolic cart (Sensormedics 2900 Z, Yorba Linda, CA, USA). Heart rate and blood pressure were measured every minute during increasing work rate exercise and recovery. A 12-lead ECG was recorded every minute. The exercise test was stopped when one or more of the following criteria were present: predicted heart rate, fatigue, dyspnoea, excessive systemic blood pressure increase ($\geq$230/130 mmHg), $\geq$2 mm ST depression in at least two adjacent leads, and/or angina. The anaerobic threshold was measured by the V-slope method.[20] Peak oxygen uptake was the average oxygen uptake during the last 15 s of exercise.

Dobutamine stress echocardiography

Under continuous ECG monitoring, dobutamine was infused into a peripheral antecubital vein at an incremental regimen of 5 μg/kg/min every 3 min until a maximum of 20 μg/kg/min. In fact, we focused on the contractile response of viable myocardium which can be determined with doses equal or below 20 μg/kg/min. Moreover, we wanted to avoid myocardial ischaemia that is habitually induced by higher doses of dobutamine. Echocardiographic studies were performed with the patients supine in the left lateral position. Two-dimensional echo images were continuously acquired from the parasternal long-axis, short-axis, and apical six-chamber views using a wide-angle mechanical scanner (2.5 MHz, Challenge, ESAOTE, Italy).

Measurements

A 16-segment model was used for LV contractility analysis. Each segment was visually graded using a semi-quantitative scoring

system, where 1 = normal, 2 = hypokinetic, 3 = akinetic, and 4 = dyskinetic. Systolic wall thickening score index (SWTI) was calculated at rest and at each stage of dobutamine infusion.[21] A 20% reduction in systolic wall thickening represents the 95% CI, discriminating a significant difference between normal and abnormal contractile response to low-dose dobutamine by two-dimensional echocardiography in our laboratory.

Data analysis

All studies were analysed with an off-line system equipped with digital processing (Panasonic AG 7700). Representative cycles of rest and peak dobutamine dose images in comparable views were digitized and positioned side-by-side on a quad-screen format. The echocardiographic images were evaluated in a blinded manner by two independent, experienced observers who adopted the same assessment criteria. Disagreement between the two observers occurred in 7% of studies. Differences in interpretation were resolved by a third independent cardiologist. In any of the following examinations, an improvement in the contractile response to dobutamine, compared with the initial study, was considered a reduction in SWTI by ≥ 1 at peak infusion and/or a $\geq 20\%$ reduction in systolic wall thickening in at least two adjacent segments.

Brachial artery vasomotor function

All studies were performed in a room with constant temperature (23°C), barometric pressure (760 millibar), and humidity (50%). Patients were evaluated in the morning in fasting condition. After 5 min of relaxation in supine position, a 7.5 MHz ultrasound probe was positioned over the dominant arm to detect good quality brachial artery images (ESAOTE Challenge, Florence, Italy). Acquisition started after fixation of the probe in a stereotaxic arm in order to avoid artefacts due to operator movements. Images were taken at baseline for 30 s, 90 s after cuff release (flow-mediated response), and 30 s after 0.3 mg sublingual nitroglycerin [endothelium-independent (EID) response] according to recommendations recently published.[22] Flow-mediated dilation was evaluated after release of a paediatric sphygmomanometer inflated at 240 mmHg for 4.5 min at the wrist. We considered a normal response as a 7% or greater increase in diameter from resting values (2 SD of the difference between repeated measurements in our research and in other laboratories).[23,24] EID evaluation was based on the percent change in diameter from baseline 5 min after nitroglycerin. Normal response for EIDBA was >10% increase in diameter from resting values.[23,24] Images were processed for analysis after digital conversion and evaluated by two independent experienced operators unaware of the clinical picture and blinded to each other's interpretation. Intraobserver and interobserver variability were assessed in 250 consecutive subjects with a variety of conditions, and results were acceptable and in agreement with those of other laboratories ($1.2 \pm 0.8\%$ and $1.9 \pm 0.9\%$, respectively).[23,24]

Exercise training

A supervised ET program was performed at the hospital's gym, three times a week for 8 weeks, as previously described.[25] Exercise intensity was chosen at 60% of peak VO_2. Each session lasted about 1 h, beginning with stretching exercise for 15 min, followed by 40 min of cycling on an electronically braked cycle ergometer (Ergometrics 800 S). Blood pressure and heart rate were measured at rest, at the end of cycling, and after 5 min of loadless recovery. Patients were recommended to avoid exercise at home during the study period.

Blood chemistry

CoQ_{10} and Vitamin E assays

CoQ_{10} was determined by HPLC using a direct extraction method, recently described.[4] Normal values for plasma CoQ_{10}, in the Italian region where the study was conducted were 0.78 ± 0.2 μg/mL, and are in agreement with findings by other authors.[26] So far, no influence of aging on this range has been found. For the vitamin E assay conditions were similar except for the column (Supelcosil LC-18, 7, 5 × 0, 46 cm, 3 μm id), mobile phase (100% methanol, flux 1.5 mL/min) and UV detection (292 nm).

Plasma lipids were measured using conventional enzymatic methods (Boheringer Mannheim, Mannheim, Germany) on a Hitachi 917 biochemical analyzer (Hitachi, Tokyo, Japan).

Statistical analysis

Data were analysed with SAS (SAS Institute, 2000) as a factorial study with a cross-over design by analysis of covariance taking into account the repeated nature of the experiment. In particular, a PROC MIXED procedure and a factorial MODEL with CoQ_{10}, ET, period and all interaction effects within a repeated statement in SAS System was carried out. The sphericity assumption was verified using the Mauchly's criterion for all variables.

The main effects of the factors and the interactions between factors were assessed by statistical models as above, and if significant, means comparison was performed by analysis of contrasts.

Regression analysis was also performed and a correlation coefficient was expressed. Statistical significance was considered at $P < 0.05$. Data is presented as mean ± SD.

Results

Of 23 patients enrolled, 21 completed the protocol. One patient dropped out after 2 months for reasons related to work, another one had an orthopaedic injury that limited his ability to exercise. There were no side effects attributed to CoQ_{10} or untoward events during training sessions in patients who completed the protocol. The Mauchly's sphericity test on orthogonal components was significant only for EID and EDD ($P < 0.05$ and $P < 0.01$, respectively). None of the variables showed a significant effect of period by treatment (CoQ_{10}, ET) interactions. Mean data ± SD for cardiopulmonary test, blood chemistry, and functional indexes are reported in *Tables 2–4*.

Factorial analysis—CoQ_{10} main effect

As shown in *Table 5*, CoQ_{10} supplementation resulted in a four-fold increase in plasma CoQ_{10} level (from 0.82 ± 0.5 μg/mL to 3.64 ± 1.8 μg/mL, $P < 0.0001$). Moreover, CoQ_{10} treatment significantly decreased plasma levels of uric acid (−3%, $P < 0.0001$) and increased HDL (+3%, $P = 0.0588$) whereas total cholesterol, LDL-C, triglycerides, and vitamin E levels did not change significantly.

CoQ_{10} supplementation significantly affected peak VO_2 (+9%, $P = 0.0001$) as reported in *Table 5*. Similarly resting LVEF significantly increased from study entry in CoQ_{10}-treated subjects (+10%, $P = 0.0023$). LVEF improved significantly also at peak dobutamine (+18%, $P < 0.0001$) in relation to a decrease in LV end-systolic volume index (from 57 ± 7 mL/m^2 to 45 mL/m^2, $P < 0.01$).

SWTI had similar improvements as EF both at rest and at peak dobutamine (−9% and −12%, respectively; $P < 0.0001$). These improvements were related to changes in regional contractility. Of 195 segments with resting wall motion abnormalities, 125 demonstrated improved contractility ($P < 0.01$ vs. initial), and these changes were evident during the first 5 min of dobutamine infusion.

Improvement in the contractile response was more evident among initially akinetic (+33%) and hypokinetic

Table 2 Cardiopulmonary exercise testing

	Study entry placebo	Q_{10}	Q_{10} + ET	Placebo	Placebo + ET
Peak VO_2 (mL/kg/min)	17.35 ± 3.6	19.6 ± 4.8	21.5 ± 4.7	17.9 ± 3.8	19.9 ± 3.7
AT VO_2 (mL/kg/min)	9.6 ± 2.3	13.5 ± 3.8	14.4 ± 3.9	9.9 ± 2.6	12.6 ± 2.8
Ventilation (L/min)	49.5 ± 15	68.9 ± 14	71.2 ± 14	46.4 ± 15	66.1 ± 12
Peak O_2 pulse (mL/beat)	9.5 ± 1.5	10.8 ± 1.6	11.8 ± 1.4	8.9 ± 2.2	9.9 ± 2
$\Delta VO_2/\Delta W$ (mL/min/W)	7.5 ± 0.8	8.3 ± 0.7	8.9 ± 0.6	7.4 ± 1.0	8.1 ± 1.0
Peak work rate (W)	108 ± 21	123 ± 20	133 ± 24	98 ± 19	120 ± 18
Resting heart rate (bpm)	74.1 ± 11	76.5 ± 12	72 ± 12	76 ± 13	75.7 ± 14
Peak heart rate (bpm)	130 ± 20	137 ± 19	142 ± 19	129 ± 20	136 ± 20
Peak systolic blood pressure (mmHg)	148 ± 22	173 ± 25	181 ± 30	142 ± 23	163 ± 15

Data is reported as mean ± SD ($n = 21$).

Table 3 Blood chemistry

	Study entry placebo	Q_{10}	Q_{10} + exercise	Placebo	Placebo + exercise
Total cholesterol (mg/dL)	196 ± 45	204 ± 43	193 ± 39	199 ± 44	184 ± 39
LDL-C (mg/dL)	115 ± 37	122 ± 34	112 ± 32	124 ± 40	107 ± 33
HDL-C (mg/dL)	55 ± 15	55 ± 21	56 ± 18	52 ± 16	53 ± 16
Triglycerides (mg/dL)	128 ± 73	129 ± 72	140 ± 99	135 ± 96	125 ± 95
Uric acid (mg/dL)	5.8 ± 1.7	5.4 ± 1.1	5.3 ± 1.3	5.6 ± 1.2	5.4 ± 1.3
Vitamin E (μg/mL)	10.3 ± 5.1	11.4 ± 4.1	10.0 ± 3.1	9.24 ± 2.7	9.56 ± 2.2
CoQ_{10} (μg/mL)	0.82 ± 0.5	3.25 ± 1.52	4.0 ± 2.1	0.83 ± 0.4	0.87 ± 0.43

Data is reported as mean ± SD ($n = 21$).

Table 4 Vasomotor reactivity, EF and SWTI

	Study entry	Q_{10}	Q_{10} + exercise	Placebo	Placebo + exercise
EDDBA (%)	3.99 ± 1.5	5.64 ± 1.95	7.53 ± 3.2	4.19 ± 1.9	5.99 ± 2.6
EIDBA (%)	13.9 ± 6.6	14.6 ± 4.6	14.7 ± 4.6	12.03 ± 5.4	16.05 ± 5.1
Resting EF (%)	37 ± 8.3	43 ± 8.7	45 ± 7.5	37.9 ± 8	43 ± 5.5
Peak EF (%)	46.7 ± 8.4	53.9 ± 9.4	61.3 ± 8	44.5 ± 8.3	53.7 ± 9.5
Resting SWTI	2.23 ± 0.3	1.96 ± 0.4	1.83 ± 0.3	2.19 ± 0.3	2.01 ± 0.3
Peak SWTI	1.86 ± 0.3	1.57 ± 0.3	1.43 ± 0.2	1.87 ± 0.3	1.59 ± 0.3

Data is reported as mean ± SD ($n = 2$).

(+25%) segments when compared with dyskinetic ones (+6%). Improvement in SWTI was correlated with changes in plasma CoQ_{10} levels ($r = -0.52$, $P < 0.01$).

The endothelium-dependent relaxation improved significantly in CoQ_{10}-treated subjects (+38%, $P = 0.0021$), whereas EID was not significantly affected by CoQ_{10} supplementation. Improvement in the endothelium-dependent relaxation after CoQ_{10} supplementation was correlated with the increase in CoQ_{10} levels ($r = 0.61$, $P < 0.01$). Patients with plasma CoQ_{10} levels above 2.4 μg/mL showed the highest improvement in endothelium-dependent dilation of brachial artery (EDDBA) (*Figure 1A*) and SWTI at peak dobutamine (*Figure 1B*) ($P < 0.01$ and $P < 0.05$, respectively).

Factorial analysis—ET main effect

As shown in *Table 6*, ET increased CoQ_{10} plasma levels even if not significantly ($P = 0.0652$). This is clearly related to a higher increase in CoQ_{10} levels when CoQ_{10} intake was associated with ET, as also indicated by the ET*CoQ_{10} interaction effect described in the next paragraph.

Moreover, ET significantly effected plasma lipid profile. In particular, ET was able to significantly reduce total cholesterol levels (−7%, $P = 0.0122$), LDL-C (−12%, $P = 0.0017$), and uric acid (−2%, $P < 0.0001$). HDL-C, triglycerides, and vitamin E levels did not change significantly.

ET produced significant improvements in cardiopulmonary indexes, as highlighted by a significant increase in peak VO_2 (+11%, $P < 0.0001$). Similarly, resting LVEF raised significantly in subjects undergoing ET (+10%, $P = 0.0007$). LVEF improved significantly also at peak dobutamine (+18% from study entry, $P < 0.0001$). SWTI showed similar improvements as EF both at rest and at peak dobutamine (−7% and −12%, respectively; $P < 0.0001$). The endothelium-dependent relaxation improved significantly from study entry in subjects undergoing ET (+46%, $P = 0.0018$). Similarly, EID was significantly affected by ET, although to a lower extent (+14%, $P = 0.0465$).

Table 5 Factorial analysis—CoQ_{10} main effect

	Coenzyme Q_{10}				*P*-value
	Yes		No		
	Mean ± SD	% var	Mean ± SD	% var	
Q_{10}	3.6 ± 1.8	346	0.9 ± 0.4	4	<0.0001
Vitamin E	10.7 ± 3.6	3	9.4 ± 2.4	−9	
Cholesterol	199 ± 40	1	192 ± 41	−2	
LDL	117 ± 32	2	116 ± 36	1	
Triglycerides	135 ± 83	5	130 ± 92	1	
HDL	56 ± 19	1	53 ± 16	−4	0.0588
Uric acid	5.3 ± 1.2	−8	5.5 ± 1.2	−5	<0.0001
SWTIp	1.5 ± 0.3	−19	1.7 ± 0.3	−7	<0.0001
SWTI	1.9 ± 0.3	−15	2.1 ± 0.3	−6	<0.0001
FEp	58 ± 9	23	49 ± 10	5	<0.0001
FE	44 ± 8	19	40 ± 8	9	0.0023
VO_2p	21 ± 5	18	19 ± 4	9	0.0001
EID	15 ± 5	5	14 ± 5	1	
EDD	6.6 ± 2.7	65	5.1 ± 2.4	27	0.0021

Table 6 Factorial analysis—ET main effect

	Exercise training				*P*-value
	Yes		No		
	Mean ± SD	% var	Mean ± SD	% var	
Q_{10}	2.4 ± 2.1	199	2.0 ± 1.6	146	0.0652
Vitamin E	9.8 ± 2.5	−6	10.3 ± 3.5	0	
Cholesterol	188 ± 37	−4	202 ± 42	3	0.0122
LDL	110 ± 31	−4	123 ± 35	8	0.0017
Triglycerides	132 ± 92	3	132 ± 81	3	
HDL	54 ± 17	−1	54 ± 18	−2	
Uric acid	5.4 ± 1.2	−7	5.5 ± 1.1	−5	<0.0001
SWTIp	1.5 ± 0.3	−19	1.7 ± 0.3	−7	<0.0001
SWTI	1.9 ± 0.3	−14	2.1 ± 0.3	−7	<0.0001
FEp	57 ± 9	23	49 ± 10	5	<0.0001
FE	44 ± 8	19	40 ± 8	9	0.0007
VO_2p	21 ± 4	19	19 ± 4	8	<0.0001
EID	15 ± 5	10	13 ± 5	−4	0.0465
EDD	68 ± 2.9	69	4.9 ± 2.0	23	0.0018

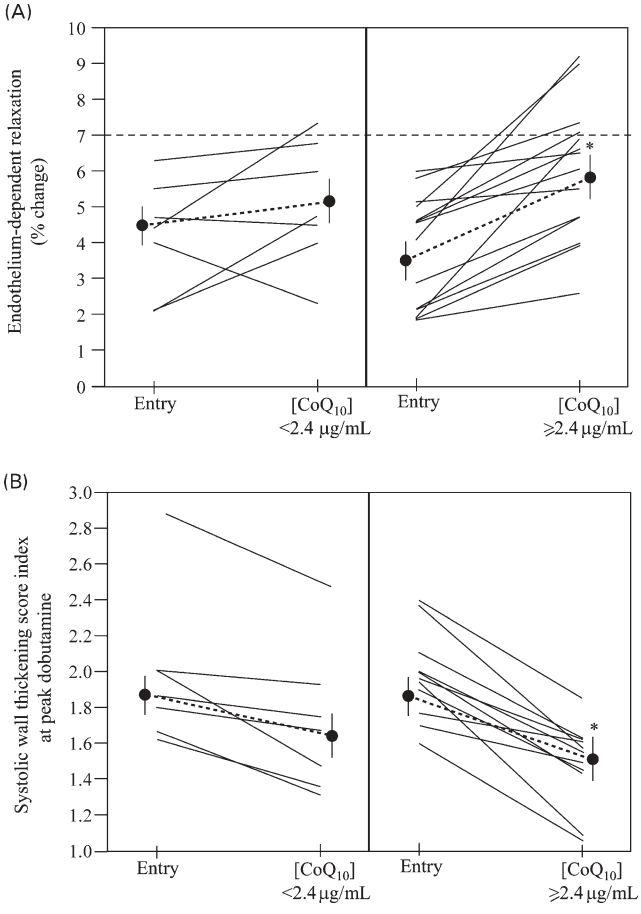

Figure 1 (*A*) Endothelium-dependent relaxation after CoQ_{10} supplementation. After CoQ_{10} supplementation, the average improvement in endothelium-dependent relaxation (dotted line) was six times greater in patients with plasma CoQ_{10} level >2.4 μg/mL ($n = 14$, group B), when compared with those with plasma CoQ_{10} level <2.4 μg/mL ($n = 7$, group A) (60.4 vs. 13.3%, *$P < 0.0001$). (*B*) SWTI at peak dobutamine infusion after CoQ_{10} supplementation. In patients with plasma CoQ_{10} >2.4 μg/mL ($n = 14$, group B) the average decrease in SWTI (dotted line) was statistically significant when compared with patients with plasma CoQ_{10} level <2.4 μg/mL ($n = 7$, group A). *$P < 0.04$ vs. A.

Table 7 Factorial analysis CoQ_{10}*ET effect

	P-interaction
SWTIp	0.01
SWTI	0.18
FEp	0.37
FE	0.17
VO_2p	0.78
EDD	0.92
Q_{10}	0.06
Uric acid	0.0001

Factorial analysis—ET CoQ_{10} interaction effect

Factorial analysis indicated a significant interaction effect of CoQ_{10} and ET in some haematic and functional indexes (*Table 7*).

A significant interaction of CoQ_{10} and ET in reducing peak systolic wall thickening ($P = 0.00192$), as well as plasma uric acid levels ($P < 0.0001$) was observed. This might be interpreted as a synergistic effect of both factors in the ability of absorbing CoQ_{10}. Moreover, no other significant interactions were evident for all the remaining functional parameters. However, for some parameters we cannot exclude a significant interaction of the two factors, as limited sample size might introduce a type II error in the analysis. In particular, even if not significant ($P = 0.0603$), higher levels of plasma CoQ_{10} in patients supplemented with CoQ_{10} while undergoing ET were observed.

Discussion

The results of the present study demonstrate that, in patients with NYHA class II and III CHF secondary to ischaemic heart disease, oral CoQ_{10} supplementation significantly improved the endothelium-dependent relaxation of the brachial artery, LV contractility, peak VO_2, and the main

parameters investigated through the cardiopulmonary test. The combination of CoQ_{10} supplementation with ET determined more marked improvements than CoQ_{10} or ET alone. Changes in the endothelium-dependent relaxation after CoQ_{10} alone or in combination with ET were correlated with changes in plasma CoQ_{10} levels.

Plasma CoQ_{10} concentration depends on several factors, including the metabolic demand of various tissues; plasma levels of 0.6–1.0 μg/mL is considered as normal.[26] In the present study, plasma levels of CoQ_{10} at study entry were within the normal range (0.82 ± 0.5 μg/mL). Supplementation with CoQ_{10} 300 mg/day, lead to a four-fold increase in plasma levels, which correlated with improved LV function. Previous studies provided conflicting results about the level that CoQ_{10} should reach in plasma in order to elicit benefits in heart failure patients. Langsjoen[17] postulated a threshold of 2.5 μg/mL, above which marked effects can be observed.

This level cannot be reached with low oral doses, and this 'threshold hypothesis' helps to explain why results obtained with 100 mg/day dosages, in advanced heart failure, were not univocal regarding the effects on LV function. In the present study, the oral dose of CoQ_{10} was three times higher and raised plasma CoQ_{10} levels well above the postulated threshold (3.25 ± 1.5 μg/mL). In the light of our results doses of 200–300 mg/day should therefore be preferred. During the trial, none of the patients received statins. Acting as HMGCoA reductase inhibitors, these drugs lower the production of mevalonate, a critical precursor for both cholesterol and CoQ_{10} synthesis. Extensive work has established the impact of statin treatment on blood and tissue levels of CoQ_{10}.[27–29] Even though the effect of statin treatment on tissue levels of CoQ_{10} is still debated,[29,30] there is no doubt that statins have a dose-related lowering effect on plasma CoQ_{10}.[27] As the aim of the present study was to investigate the relationship between CoQ_{10} treatment, CoQ_{10} plasma levels, and endothelial and cardiac function, we chose to exclude patients on statin treatment, in order to avoid a possible bias. On the basis of the known pleiotropic effects of statins, we cannot exclude that the addition of statins to our therapeutic schemes could have generated even better results.

A second important element is the antioxidant activity of CoQ_{10}.[2] Antioxidant properties are related to a direct antioxidant effect of ubiquinol and to the capability of regenerating Vitamin E from tocopheryl radical.[31,32] Moreover, CoQ_{10} might improve nitric oxide bioactivity by decreasing superoxide generation and by interacting with superoxide generation and free radicals.[33] In conditions of high oxidative stress, such as CHF and the presence of multiple coronary risk factors, the rate of inactivation of nitric oxide to peroxynitrite by superoxide anions may be reduced by CoQ_{10}, which can also protect against nitrosative damage.[34] CoQ_{10} may also influence vascular function indirectly via inhibition of oxidative damage to LDL.[35] Moreover, CoQ_{10} supplementation improved endothelial function in dyslipidaemic patients with type II diabetes, and this improvement was associated with higher plasma CoQ_{10} levels (from 1.3 to 4.8 mmol/L).[36] In the present study, we found similar results. The endothelium-dependent relaxation improvement was correlated with changes in plasma CoQ_{10} concentration.

Even though the improvements in the functional parameters were more pronounced when the patients underwent both CoQ_{10} supplementation and ET, factorial analysis showed a clear synergistic effect of CoQ_{10} only for peak SWTI suggesting that ET amplifies the already described effect of CoQ_{10} on contractility of dysfunctional myocardium. Moreover, plasma level of CoQ_{10} itself resulted synergistically affected by CoQ_{10} and ET. ET might therefore increase bioavailability of CoQ_{10}.

Finally, the high plasma levels of CoQ_{10} were not associated with side effects. Both the improved ATP production and the antioxidant properties may be involved in explaining these benefits. We found a significant improvement in LV contractility in dysfunctional segments located in non-infarcted areas served by stenotic arteries, where hibernation and/or chronic stunning is likely to occur. The upregulation of contractile function after CoQ_{10} suggests that chronic post-ischaemic stunned cells improve or normalize their metabolism and function.[37] This effect translates into mechanical efficiency and contributes to reduce LV dysfunction. It is noteworthy that this effect was obtained without any change in heart rate, differently from traditional inotropic substances. There is evidence that exercise, by increasing shear stress, stimulates e-NOS expression and nitric oxide synthesis. In turn, CoQ_{10} might reduce nitric oxide inactivation through the previously cited action of superoxide scavenging. Moreover, CoQ_{10} supplementation was found to upregulate guanylyl cyclase, the receptor for nitric oxide, in human skeletal muscle.[38] Furthermore, the improvement in LV contractility after ET has been related to enhanced coronary collateralization, which is modulated by nitric oxide.[39,40]

We did not use pharmacological compounds, acetylcholine and *N*-monomethyl-L-arginine, to study the effects of CoQ_{10} alone or in combination with ET on the endothelial function. However, it has been recently demonstrated that the method we used is sufficiently accurate to monitor vasomotor reactivity of conduit arteries,[22] even though, we cannot extrapolate the results observed in the brachial artery to smaller arteries or microcirculation, because mediators involved in vasomotor reactivity are different.[24] Low-dose dobutamine was used to detect changes in myocardial contractility after ET in humans with ischaemic cardiomyopathy, showing good agreement with thallium imaging.[21]

In conclusion, in patients with ischaemic cardiomyopathy and CHF in NYHA functional class II and III, oral supplementation with CoQ_{10} at doses that increase plasma CoQ_{10} levels four-fold from study entry was safe and determined significant improvements in EDDBA, LV contractility, and functional capacity. The addition of ET to oral CoQ_{10} led to further elevation in plasma CoQ_{10} levels, and was associated with more marked improvements in all the above cited parameters. These potential benefits were not accompanied by side effects.

Conflict of interest: none declared.

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