

# Effect of coenzyme Q<sub>10</sub> administration on endothelial function and extracellular superoxide dismutase in patients with ischaemic heart disease: a double-blind, randomized controlled study

Luca Tiano<sup>1\*</sup>, Romualdo Belardinelli<sup>2</sup>, Paola Carnevali<sup>3</sup>, Federica Principi<sup>1</sup>,  
Giovanna Seddaiu<sup>4</sup>, and Gian Paolo Littarru<sup>1</sup>

<sup>1</sup>Institute of Biochemistry, Polytechnic University of the Marche, Via Ranieri, 60100 Ancona, Italy; <sup>2</sup>Lancisi Heart Institute, Azienda Ospedali Riuniti, Ancona, Italy; <sup>3</sup>Salesi Paediatric Hospital, Polytechnic University of the Marche, Ancona, Italy; and <sup>4</sup>Department of Agronomical Sciences and Agronomical Vegetal Genetics, University of Sassari, Sassari, Italy

Received 30 June 2006; revised 30 May 2007; accepted 7 June 2007; online publish-ahead-of-print 19 July 2007

## KEYWORDS

Coenzyme Q<sub>10</sub>;  
Extracellular superoxide  
dismutase;  
Endothelium-dependent  
vasorelaxation

**Aims** This randomized controlled study was designed to determine whether oral coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) supplementation (100 mg tid) was able to improve extracellular superoxide dismutase (ecSOD) activity and endothelium-dependent (ED) vasodilation in patients with coronary artery disease (CAD). ecSOD, a major antioxidant enzyme system of the vessel wall, is reduced in patients with CAD. Moreover, there is a strong correlation between endothelium-bound ecSOD and the ED dilation of conduit arteries. CoQ<sub>10</sub> has been recently shown to improve the ED relaxation in diabetic patients.

**Methods and results** Thirty-eight CAD patients (33 M/5 F, mean age 55 ± 4 years, ejection fraction 57.5 ± 8%) were randomized into two groups. One group (n = 19) received CoQ<sub>10</sub> orally at doses of 300 mg/day for 1 month, whereas the other group received a placebo. On entry and after 1 month, all patients underwent brachial artery ED assessment, cardiopulmonary exercise test, and the measurement of endothelium-bound ecSOD activity. A total of 33 patients completed the study. ecSOD, ED relaxation, as well as peak VO<sub>2</sub> and O<sub>2</sub> pulse increases in the CoQ<sub>10</sub>-treated group were statistically greater vs. the variations in the placebo group. In particular, improvements elicited by CoQ<sub>10</sub> supplementation were remarkable in subjects presenting low initial endothelium-bound ecSOD and thus more prone to oxidative stress.

**Conclusion** Improvements in the ED relaxation and endothelium-bound ecSOD activity might be related to CoQ<sub>10</sub> capability of enhancing endothelial functionality by counteracting nitric oxide oxidation. The enhancement of peak VO<sub>2</sub> and of O<sub>2</sub> pulse is likely due to the bioenergetic effect of CoQ<sub>10</sub>; on the other end, the improved VO<sub>2</sub> could also depend on the observed enhanced peripheral endothelial function.

## Introduction

Extracellular superoxide dismutase (ecSOD), discovered by Marklund and co-workers in 1982,<sup>1,2</sup> is a different enzyme from the two well-known intracellular superoxide dismutases, namely Cu,ZnSOD and MnSOD. It is a tetramer containing one copper and one zinc atom per subunit, which are required for enzymatic activity; the location of ecSOD gene in humans is chromosome 4q21 and shows a 60% homology with Cu,ZnSOD but minimal homology with MnSOD.<sup>3</sup> The human mRNA for the enzyme is highly expressed in heart, placenta, pancreas, and lung, and in lower levels in kidney, skeletal muscle, and liver.

The primary location of ecSOD in tissues is in the extracellular matrix and on cell surfaces, where its concentration

is 20 times higher than in plasma. The association of ecSOD with the tissue is accomplished by a heparin-binding domain, which recognizes heparan-sulfate proteoglycans on cell surface and in the matrix. In fact, intravenous injections of heparin in humans and other species lead to an immediate increase in plasma ecSOD content,<sup>4</sup> allowing the determination of endothelium-bound ecSOD in humans *in vivo*.<sup>5</sup> The highest tissue concentrations are found in blood vessels, lung, kidney, and uterus.<sup>6</sup>

The physiopathological role of ecSOD has been examined in vascular-related diseases, atherosclerosis, hypertension, diabetes, ischaemia-reperfusion injury, lung disease, various inflammatory conditions, and neurological diseases.<sup>3</sup>

Landmesser *et al.*<sup>5</sup> showed that vascular ecSOD activity is substantially reduced in patients with coronary artery disease (CAD). Moreover, a strong correlation was found between the endothelium-bound ecSOD and the flow-dependent

\* Corresponding author. Tel: +39 071 2204394; fax +39 071 2204398.  
E-mail address: luca.tiano@unicam.it

endothelial-mediated dilation (FMD), a functional parameter commonly used as a biomarker of vascular function.

Coenzyme Q<sub>10</sub>, commonly referred to as CoQ<sub>10</sub> in humans, where the homologue with 10 isoprenoid units is the predominant one, has a well acknowledged antioxidant role, besides its deeply inquired function in mitochondrial bioenergetics.

Several studies have demonstrated that CoQ<sub>10</sub> positively affects heart performance in congestive heart failure and ischaemic heart disease.<sup>7</sup> Moreover, CoQ<sub>10</sub> is also endowed with hypotensive action.<sup>8,9</sup> These effects are usually ascribed to CoQ<sub>10</sub> ability to improve mitochondrial bioenergetics and to counteract oxidative stress in the myocytes. Recent studies have also demonstrated an improvement in the endothelial function after treatment with CoQ<sub>10</sub>.<sup>10,11</sup> In particular, Watts *et al.*<sup>10</sup> showed that CoQ<sub>10</sub> attenuates the endothelial dysfunction in patients with type II diabetes mellitus. Recently, the data from our group<sup>11</sup> highlighted that CoQ<sub>10</sub> greatly attenuates the endothelial dysfunction in patients affected by ischaemic heart disease; in the same patients, CoQ<sub>10</sub> was also capable of significantly improving peak VO<sub>2</sub>.

Nitric oxide (NO) plays a critical role in the endothelial homeostasis. It is involved in the regulation of arterial blood pressure and also shows antiatherogenic properties. Oxidative stress has known repercussions on the bioavailability of NO,<sup>12</sup> which, among other actions, is able to induce the ecSOD expression. Therefore, antioxidant substances capable of counteracting NO inactivation may improve the endothelial dysfunction through an increase in the ecSOD activity.

This randomized controlled study was primarily designed to determine whether the oral CoQ<sub>10</sub> supplementation (100 mg tid) was able to improve the ecSOD activity and the endothelium-dependent (ED) vasodilation in patients with CAD. The pathophysiological relevance of the endothelial dysfunction is well known and the ameliorating endothelial-dependent vasorelaxation, also through an improved ecSOD activity, would likely result in improved cardiovascular function and a better prognosis for these patients.

## Methods

### Patients

This study has been performed with the subjects' written informed consent. The patients were included if they had had cardiac events (CABG, PTCA/myocardial infarction) 3 months or more before the enrollment, were clinically stable, able to exercise, and their medications regime had not been changed in the 3 months preceding the study. The exclusion criteria were involvement in cardiac rehabilitation programmes and/or antioxidant supplementation.

A minimum sample size of 32 patients was used in order to have a probability of 80% to detect a treatment difference at a two-sided 5% significance level and taking into account the main endpoints of the study, i.e. the effects of CoQ<sub>10</sub> on endothelium-bound ecSOD and brachial artery ED vasodilation. For this purpose, we assumed a standard deviation of 4.8 U/mL plasma/min and 1.9% and a smallest worthwhile change of 5 and 2 U, respectively. Forty patients were initially assessed as eligible; two of them were excluded from enrolment since they did not meet the inclusion criteria. The 38 enrolled patients underwent a block randomization, using a computer-generated sequence: 19 were allocated in the intervention group and 19 in the placebo group. All the 19 patients

belonging to the intervention group completed the study and were analysed. Within the placebo group, two patients were lost to the follow-up, one for personal reasons and the other for orthopaedic injury. Moreover, three patients from the same group completed the study but were excluded from the analysis, as the degree of haemolysis in plasma did not allow to quantify ecSOD. Overall, 19 patients were evaluated in the intervention group and 14 in the placebo group.

The patients in the intervention group were treated with CoQ<sub>10</sub> (Jarrow Formulas, Los Angeles, CA) at doses of 100 mg tid for 1 month, whereas controls received a placebo (also tid). The allocation of the patients to the intervention or placebo group was concealed to the patients, to the personnel conducting the clinical tests, and to the laboratory people involved in the analysis. The codes were kept by the nurse who dispensed the bottles with CoQ<sub>10</sub> or placebo and were broken when the trial was over and patients had completed the tests. The details on the conventional therapy followed at the time of the study are reported in *Table 1*. The medications were not changed during the study. In particular, beta-blockers were not suspended before the cardiopulmonary test. For all the medications, including CoQ<sub>10</sub>, the last dose was taken the evening before the test. On entry and after 1 month, all the patients underwent the brachial artery ED assessment, cardiopulmonary exercise testing, and the measurement of endothelium-bound ecSOD activity.

The primary endpoints of this study were to determine the effect of the CoQ<sub>10</sub> administration on heparin-released endothelium-bound ecSOD and on brachial artery vasomotility. A secondary endpoint was to determine the effect of CoQ<sub>10</sub> on the cardiopulmonary test.

### Determination of endothelium-bound extracellular superoxide dismutase

ecSOD is specifically released from the endothelium into plasma by heparin bolus injection, allowing the determination of the ecSOD activity in humans *in vivo* without affecting plasma Cu-, Zn-SOD or Mn-SOD activity.<sup>4,13</sup> For the measurement of plasma ecSOD activity, the patients were sampled using a cannula in the cubital vein. Two baseline venous blood samples in EDTA-containing vacuum tubes were obtained; subsequently, 5000 U of heparin was injected and blood samples were obtained from the cannula 1, 3, 5, 7, and 10 min after heparin injection. For each sample, the first 2 mL of blood were discarded. The blood samples were kept on ice and centrifuged within 2 h at 2000 g for 15 min at 4°C. Plasma was then stored at -80°C until assayed. The activity of SOD was measured at pH 8.2 by a modified nitrite method.<sup>14</sup> Superoxide generated by hypoxanthine and xanthine oxidase was changed to nitrite ion by hydroxylamine. Nitrite ion was measured spectrophotometrically at 550 nm by the use of a chromogen. The amount of SOD required to inhibit the rate of nitrite ion generation by 50% was defined as 1 U of SOD activity.<sup>15</sup> The calibrations were performed with known amounts of purified bovine SOD; the ecSOD activity (U/mL/min) was finally calculated as the area under the curve of the increase of the plasma SOD activity within 10 min after heparin injection. (*Figure 1*).

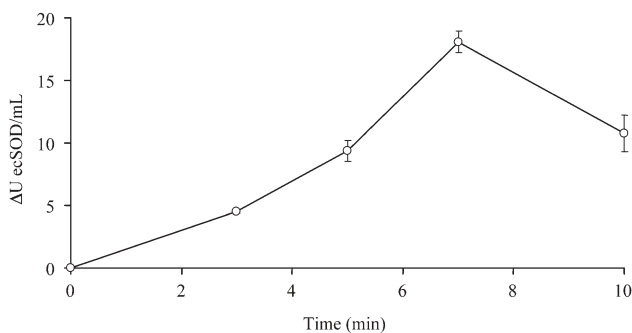
### Brachial artery vasomotor function (flow-dependent endothelial-mediated dilation)

All the studies were performed in a room with constant temperature (23°C), barometric pressure (760 mbar), and humidity (50%). The patients were evaluated in the morning in fasting condition. After 5 min of rest 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 the fixation of the probe in a stereotaxic arm in order to avoid artefacts due to operator movements. The images were taken at baseline for 30 s, 90 s after the cuff release (flow-mediated response) and 30 s after the 0.3 mg sublingual

**Table 1** Clinical characteristics

	CoQ <sub>10</sub>	Controls	P-value
Males (n)	19 (17)	14 (12)	
Age (years)	54.8 ± 2.9	56.0 ± 1.7	0.73
Ejection fraction (%)	56 ± 2.1	59 ± 1.8	0.75
Peak VO <sub>2</sub> (mL/kg/min)	20.5 ± 1.1	22.5 ± 0.9	0.14
Plasma CoQ <sub>10</sub> (µg/mL)	0.63 ± 0.04	0.65 ± 0.03	0.63
FMD (%)	4.6 ± 0.7	4.3 ± 0.5	0.77
ecSOD (U/mL/min)	17.3 ± 1.9	16.6 ± 1.3	0.74
Coronary risk profile (%)			
Hypertension	11 (58)	8 (57)	0.96
Hypercholesterolaemia	11 (58)	9 (64)	0.71
Smoking	2 (11)	1 (7)	0.74
Diabetes	3 (16)	3 (21)	0.68
Medications (%)			
BB	11 (58)	12 (86)	0.09
ACEI	11 (58)	9 (64)	0.71
ASA	14 (74)	12 (86)	0.40
CaA	6 (32)	4 (36)	0.85
Statins	13 (68)	13 (93)	0.09

Clinical profile of the patients enrolled in the study. Quantitative data are reported as means ± SD. Comparisons of means were performed using unpaired *t*-test. Categorical data are reported as the number of patients and the percentage of the total population (in parentheses). Comparisons among groups were done using  $\chi^2$  test. BB, beta-blockers; ACEI, angiotensin-converting enzyme inhibitors; ASA, acetylsalicylic acid; CaA, calcium channel blocker.



**Figure 1** Pattern of the plasmatic extracellular superoxide dismutase activity in a single patient, following heparin bolus injection of 5000 U. Data are means ± SD of three replicate measurements for each time point withdrawal.

nitroglycerin administration (endothelium-independent response) according to the recommendations recently published.<sup>16</sup> The FMD was evaluated after the release of a paediatric sphygmomanometer inflated at 240 mmHg for 4.5 min at the wrist. We considered a 7% or greater increase in diameter from resting values as normal response (2 SD of the difference between repeated measurements in our research and in other laboratories).<sup>16,17</sup> The images were processed for the analysis after the digital conversion and the arterial diameter was evaluated by two independent experienced operators unaware of the clinical picture and blinded to each other's interpretation. Intra-observer variability and interobserver variability were assessed in 250 consecutive subjects with a variety of conditions, and the results were acceptable and in agreement with those of other laboratories (1.2 ± 0.8% and 1.9 ± 0.9%, respectively).<sup>16,17</sup>

### Cardiopulmonary exercise testing

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 (COSMED Quark PFT, Pavona di Albano, Italy). Heart rate and

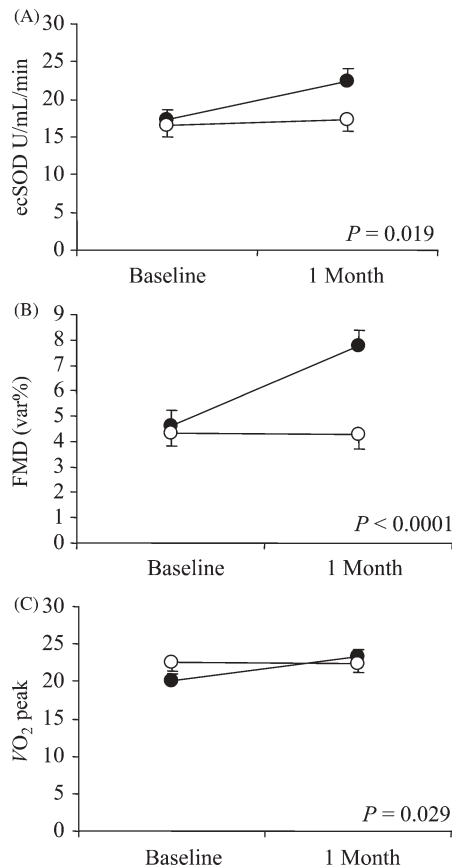
blood pressure were measured every minute during increasing work rate exercise and during recovery. A 12-lead ECG was recorded every minute. The established criteria for stopping the exercise test were the presence of one or more of the following conditions: predicted heart rate, fatigue, dyspnoea, excessive systemic blood pressure increase ( $\geq 230/130$  mmHg),  $\geq 2$  mm ST-depression in at least two adjacent leads and/or angina, a decrease in blood pressure (more than 10 mmHg) with increasing work rate, and complex ventricular arrhythmias. The peak oxygen uptake was the average oxygen uptake during the last 15 s of exercise.

### Statistical methods

The data were analysed with SAS (SAS Institute, 2000) according to a randomized controlled trial by the analysis of covariance, taking into account the repeated nature of the experiment. In particular, the PROC MIXED procedure within a REPEATED statement in the SAS system was carried out. The assumption of sphericity was verified by Mauchly's criterion for all variables. Normal distribution of data was verified using Shapiro-Wilk's test. Means and SEM have been presented for all the tested parameters. The comparison of patients in the experimental and the placebo group to verify balanced randomization was verified using unpaired *t*-test for means and  $\chi^2$  test for categorical data. The variations in the experimental and in the placebo group were compared using the ANOVA test for repeated measurements with two trials plus a between-subject effect. Two-sided *P*-values < 0.05 were deemed to be statistically significant. The Pearson correlation coefficient was calculated to assess the strength of relationship between parameters.

### Results

CoQ<sub>10</sub> supplementation resulted in a four-fold increase in plasma CoQ<sub>10</sub> level from baseline (from 0.63 ± 0.03 to 2.79 ± 0.34 µg/mL, *P* < 0.0001). No changes were observed



**Figure 2** Variations in the endothelium-bound extracellular superoxide dismutase (A), flow-mediated dilation (B), and  $\dot{V}O_2$  peak (C) in CoQ<sub>10</sub>-treated patients (black) and placebo (white). Data are reported as mean values  $\pm$  SEM at study entry (baseline) and after treatment (1 month).

in the control group. The ecSOD activity raised from  $17.3 \pm 1.7$  to  $22.4 \pm 1.3$  U/mL/min in the treated group, whereas there was only a slight change in the placebo group, from  $16.6 \pm 1.6$  to  $17.3 \pm 1.6$  U/mL/min. The ecSOD increase in the CoQ<sub>10</sub>-treated group was statistically higher vs. the variation in the placebo group, considering time point and treatment interaction ( $P = 0.019$ ) (Figure 2). Furthermore, the increase in the ecSOD activity was more pronounced in the subgroup of patients, with the lowest values on study entry. In fact, only in the subset of patients with a basal value below the median value of 17.2 U/mL/min, the increase after CoQ<sub>10</sub> administration was significant vs. the corresponding variation in the controls ( $P = 0.017$ ) (Figure 3).

### Endothelium-dependent relaxation

The initial FMD values in the overall population study were strongly correlated with the endothelium-bound ecSOD activity ( $r = 0.626$ ;  $P < 0.01$ ), and this is in agreement with similar observations reported in the literature.<sup>5</sup> The ED relaxation was improved in the CoQ<sub>10</sub> group, from  $4.6 \pm 0.6$  to  $7.8 \pm 0.6\%$ , whereas there was no change in the control group (initial:  $4.3 \pm 0.6\%$ ; one month:  $4.3 \pm 0.5\%$ ). The change in the CoQ<sub>10</sub>-treated group was highly significant when compared with the corresponding variation in the controls, considering time point and treatment interaction

( $P < 0.0001$ ) (Figure 2). The enhancement of the ED relaxation was significantly correlated with the increases in the plasma CoQ<sub>10</sub> levels ( $r = 0.71$ ,  $P < 0.0001$ ) (Figure 4).

Also for the FMD, the changes were more significant in the subgroup, with ecSOD below 17.2 U/mL/min ( $P < 0.0001$ ) (Figure 3). A correlation was found between the increases in ecSOD and the percent change in the brachial artery diameter ( $r = 0.78$ ,  $P < 0.0001$ ) (Figure 5). There were no changes in the resting brachial artery diameter at the end of the study compared with entry in all patients.

### Cardiopulmonary exercise test

At the beginning and after 1 month, the reasons for terminating the exercise tests were similar, that is, 70% had leg fatigue and 30% dyspnoea in both groups. The patients reached an average peak heart rate equal to 74% of maximal heart rate ( $220 - \text{age}$ ). As shown in Table 2, patients treated with CoQ<sub>10</sub> had significant improvements, compared with placebo, in peak  $\dot{V}O_2$  (15%), ventilatory threshold (24.5%),  $O_2$  pulse at peak exercise (21.9%),  $D\dot{V}O_2/DW$  slope (13.3%), and systolic blood pressure at peak exercise (18.6%). In particular, although, overall, patients treated with CoQ<sub>10</sub> showed a significant ( $P = 0.029$ ) increase in peak  $\dot{V}O_2$  compared with the variation measured for the control patients (Figure 2), when the patients were divided into subgroups according to low and normal basal endothelium-bound SOD levels, the variation in peak  $\dot{V}O_2$  was significant only for the subgroup starting with low ecSOD (Figure 3).

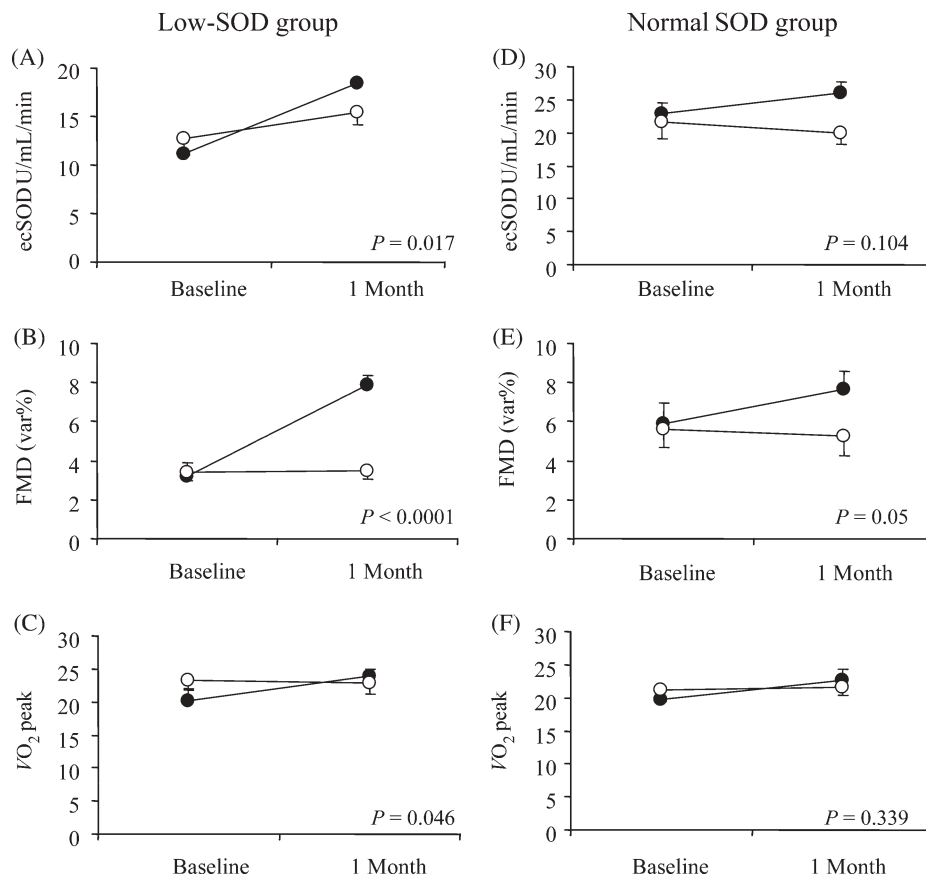
In the treated patients, at peak exercise, the respiratory exchange ratio and ventilatory equivalents for oxygen were  $1.23 \pm 0.03$  and  $39 \pm 0.8$ , respectively, at the initial evaluation, whereas the final evaluation showed values of  $1.26 \pm 0.03$  and  $41 \pm 0.9$ . Similar results were observed in the control group at peak exercise.

The peak  $O_2$  pulse increased from a basal value of  $10.9 \pm 0.5$  to  $12.5 \pm 0.6$  mL/beat after the CoQ<sub>10</sub> treatment, whereas the corresponding change in the controls was from  $10.5 \pm 0.8$  to  $10.4 \pm 0.9$  mL/beat. Also for the  $O_2$  pulse, ANOVA highlighted a significant difference ( $P < 0.05$ ) in the improvements in the CoQ<sub>10</sub> group vs. the corresponding change in the control group.

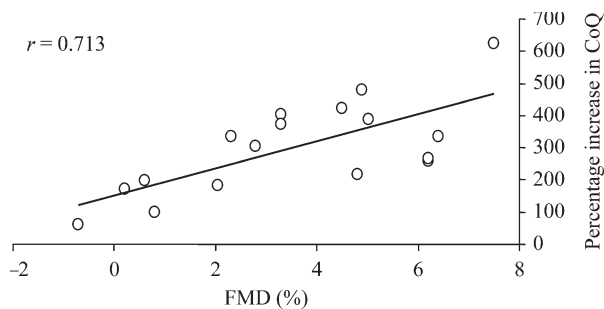
### Discussion

The results of the present investigation indicate that the oral CoQ<sub>10</sub> supplementation in CAD patients has beneficial effects, which can be ascribed either to the bioenergetic role of the quinone or to its antioxidant properties.

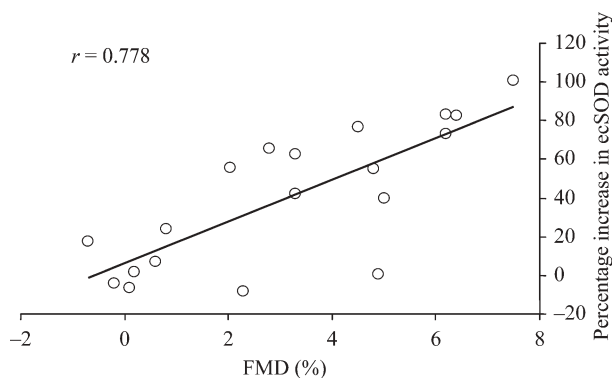
Concerning the bioenergetic aspect, a significant improvement in the peak  $\dot{V}O_2$  and in the  $O_2$  pulse was observed in the group supplemented with CoQ<sub>10</sub> compared with the change in the placebo group. This finding could be the result of the well-known role of CoQ<sub>10</sub> in the oxidative phosphorylation. In fact, the physiological concentration of CoQ<sub>10</sub>, at least in the bovine heart mitochondria, was shown to be not kinetically saturating<sup>18</sup>; therefore, it was hypothesized that even a small increase in the concentration of CoQ<sub>10</sub> in these membranes could somehow affect the mitochondrial respiratory function. Recent experimental evidence in humans<sup>19</sup> pointed out that mitochondria isolated from the atrial appendages of the patients undergoing cardiac surgery pre-treated



**Figure 3** Subpopulation analysis, low-SOD patients (below the median value: A, B, C) and normal SOD patients (above the median value: D, E, F). Variations in the endothelium-bound ecSOD (A, D), flow-mediated dilation (B, E), and  $\dot{V}O_2$  peak (C, F) in CoQ<sub>10</sub>-treated patients (black) and placebo (white). Data are reported as mean values  $\pm$  SEM at study entry (baseline) and after treatment (1 month).



**Figure 4** Correlation between percentage increase in plasmatic concentration of CoQ<sub>10</sub> and flow-mediated dilation.



**Figure 5** Correlation between percentage increase in the ecSOD activity and the flow-mediated dilation in CoQ<sub>10</sub>-treated patients.

with CoQ<sub>10</sub> had higher levels of quinone, compared with placebo controls, and a better oxidative phosphorylation efficiency. This hypothesis has also been validated *in vitro* with lymphocytes isolated from patients affected by oxidative phosphorylation disorders, where a dose-dependent increase in ATP synthesis upon incubation with CoQ<sub>10</sub> was demonstrated.<sup>20</sup> Regarding the *in vivo* aspects, besides our original observations that the CoQ<sub>10</sub> administration improves the peak  $\dot{V}O_2$  in normal and diseased people,<sup>21</sup> studies by phosphorous magnetic resonance spectroscopy have shown that the treatment with CoQ<sub>10</sub> improves the efficiency of mitochondrial respiration in patients affected by mitochondrial cytopathies.<sup>22</sup> Moreover, recent data from our group<sup>11</sup> demonstrated that the CoQ<sub>10</sub> administration improves cardiac contractility in ischaemic heart disease patients, measured by low dobutamine stress echocardiography. Since the  $\dot{V}O_2$  pulse reflects with a good approximation stroke volume, its improvement after CoQ<sub>10</sub> may imply an enhanced left ventricular performance allowing a more active life style, improved functional capacity, and ED relaxation. On the other end, the improved  $\dot{V}O_2$  could also depend on the observed enhanced peripheral endothelial function. The fact that the  $\dot{V}O_2$  improvement was significant only in the subgroup with basal low ecSOD values might indicate that the increased  $\dot{V}O_2$  peak is associated, at least in part, with the antioxidant properties of CoQ<sub>10</sub>.

The results of the present study also highlighted that the supplementation with CoQ<sub>10</sub> significantly affects the

**Table 2** Cardiopulmonary exercise testing results

	CoQ <sub>10</sub>		Placebo	
	Baseline	1 Month	Baseline	1 Month
Peak VO <sub>2</sub> (mL/kg/min)	20.0 ± 0.9	23.3 ± 1.0*	22.5 ± 1.0	22.3 ± 1.1
AT VO <sub>2</sub> (mL/kg/min)	10.6 ± 0.8	13.2 ± 0.8*	10.9 ± 0.8	10.5 ± 0.9
Ventilation (L/min)	69.5 ± 3.4	77.9 ± 3.2*	72.4 ± 4.0	71.8 ± 4.0
Peak O <sub>2</sub> pulse (mL/beat)	10.5 ± 0.4	12.8 ± 0.4*	10.9 ± 0.6	10.7 ± 0.6
ΔVO <sub>2</sub> /ΔW (mL/min/W)	7.5 ± 0.2	8.5 ± 0.2*	7.4 ± 0.3	7.4 ± 0.3
Peak work rate (W)	118 ± 4.8	143 ± 4.6*	122 ± 5.1	119 ± 5.1
Resting heart rate (b.p.m.)	78.5 ± 2.5	76.5 ± 2.8	76 ± 3.5	77 ± 3.5
Peak heart rate (b.p.m.)	135 ± 4.6	144 ± 4.4	138 ± 5.3	137 ± 5.3
Peak systolic blood pressure (mmHg)	145 ± 4.8	172 ± 5.5*	148 ± 6.1	142 ± 6.1
RER	1.23 ± 0.03	1.26 ± 0.03	1.21 ± 0.03	1.21 ± 0.03

Results of the cardiopulmonary exercise test in CoQ<sub>10</sub>-treated patients and in placebo at study entry and after 1 month. Data are reported as means + SEM. Comparisons of means were performed using unpaired *t*-test.

\**P* < 0.05.

endothelium-bound ecSOD activity, which was chosen as a relevant endpoint for its recognized role in the regulation of the vascular redox state in the extracellular space.<sup>23</sup> Moreover, the CoQ<sub>10</sub> treatment improved the ED vasodilation, which is known to have important prognostic implications.<sup>24</sup> Both effects could be related to the antioxidant properties of CoQ<sub>10</sub>, which could protect NO from the oxidative inactivation. Moreover, a dose-dependent response could be involved. In fact, both effects were obtained by raising the plasma CoQ<sub>10</sub> levels above 2.5 μg/mL, which had already been considered a threshold for obtaining cardiovascular benefits.<sup>25</sup> In the present study, a strong correlation was found between the increased plasma levels of CoQ<sub>10</sub> and the improvement in the ED brachial artery relaxation (*Figure 4*). Although the effect of CoQ<sub>10</sub> on the ED relaxation has already been shown in patients with type II diabetes<sup>10</sup> and ischaemic heart disease,<sup>11</sup> the effect of CoQ<sub>10</sub> on raising ecSOD levels represents a novel observation. In basal conditions, we showed a significant correlation between the ecSOD activity and the brachial artery ED relaxation, which is in good agreement with the data reported by Landmesser *et al.*<sup>5</sup> Furthermore, we showed a significant correlation between the increase in the endothelial-bound ecSOD activity and the improvement in the FMD after the CoQ<sub>10</sub> supplementation (*Figure 5*). The increase in the ecSOD activity as well as the improvement in the brachial artery ED relaxation were more pronounced in the subgroup of patients with the low ecSOD levels on entry. These data suggest that CoQ<sub>10</sub> is more effective in patients with decreased levels of antioxidant defences, presumably more prone to oxidative stress. Similarly, Landmesser *et al.*<sup>5</sup> found an inverse correlation between the ecSOD basal activity and the increase in the brachial artery ED relaxation after vitamin C administration. The effect of antioxidants on vascular function is therefore more pronounced in patients with a higher extent of oxidative stress, and the influence of CoQ<sub>10</sub> on the levels of ecSOD is likely the result of its antioxidant properties. We can reasonably hypothesize that CoQ<sub>10</sub> antagonizes NO inactivation to peroxynitrite, therefore making NO more available for its biological function. The availability of more NO could also lead to increased ecSOD gene-expression,<sup>23</sup> which could in turn contribute to preserve NO from inactivation by O<sub>2</sub><sup>-</sup>. Previous work

conducted in our group showed that CoQ<sub>10</sub> antagonizes *in vitro* O<sub>2</sub><sup>-</sup> produced by different biological systems.<sup>26</sup> We later interpreted these data in the light of a SOD-mimic effect as demonstrated in short-chain ubiquinone analogues by means of EPR techniques.<sup>27</sup> Therefore, CoQ<sub>10</sub>, by subtracting O<sub>2</sub><sup>-</sup> to the reaction with NO, could prevent the oxidation of NO to peroxynitrite. Furthermore, recent data reveal that CoQ<sub>10</sub> affects the expression of genes involved in human cell signalling, metabolism, and transport<sup>28</sup>; Therefore, a direct ecSOD gene induction cannot be ruled out.

We did not use pharmacological compounds—acetylcholine and *N*-monomethyl-*L*-arginine—to study the effects of CoQ<sub>10</sub> 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.<sup>29</sup> Moreover, we cannot extrapolate the results observed in the brachial artery to smaller arteries or microcirculation, because mediators involved in vasomotor reactivity are different.<sup>30</sup> It is known though that the endothelial dysfunction of peripheral arteries correlates well with that of the coronary arteries, namely the one assessed as vasomotor responses to bradykinin.<sup>31</sup> On the basis of those findings, we could reasonably hypothesize that the improvement of FMD is accompanied by a corresponding enhancement of the coronary artery vasodilation.

In conclusion, in patients with CAD with normal left ventricular function, the oral CoQ<sub>10</sub> supplementation increased the ecSOD levels and improved the ED relaxation of the brachial artery. Patients with lower levels of ecSOD had greater improvements, suggesting that the higher the oxidative stress the greater the improvement in the ED relaxation after the administration of a compound with antioxidant properties such as CoQ<sub>10</sub>. Further studies are needed to find how these effects correlate with clinical benefits.

**Conflict of interest:** none declared.

## References

1. Marklund SL, Hellner L. Superoxide dismutase in extracellular fluids. *Clin Chim Acta* 1982;126:41–51.

2. Marklund SL, Bjelle A, Elmqvist LG. Superoxide dismutase isoenzymes of the synovial fluid in rheumatoid arthritis and in reactive arthritides. *Ann Rheum Dis* 1986;**45**:847-851.
3. Fattman CL, Schaefer LM, Oury TD. Extracellular superoxide dismutase in biology and medicine. *Free Radic Biol Med* 2003;**35**:236-256.
4. Adachi T, Yamada H, Futenma A, Kato K, Hirano K. Heparin-induced release of extracellular-superoxide dismutase form (V) to plasma. *J Biochem (Tokyo)* 1995;**117**:586-590.
5. Landmesser U, Merten R, Spiekermann S, Buttner K, Drexler H, Hornig B. Vascular extracellular superoxide dismutase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. *Circulation* 2000;**101**:2264-2270.
6. Marklund SL. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. *Biochem J* 1984;**222**:649-655.
7. Mortensen SA. Overview on coenzyme Q<sub>10</sub> as adjunctive therapy in chronic heart failure. Rationale, design and end-points of 'Q-symbio'-a multinational trial. *Biofactors* 2003;**18**:79-89.
8. Digiesi V, Cantini F, Oradei A, Bisi G, Guarino GC, Brocchi A, BELLand F, Mancini M, Littarru GP. Coenzyme Q<sub>10</sub> in essential hypertension. *Mol Aspects Med* 1994;**15**(suppl.):s257-s263.
9. Lonnrot K, Porsti I, Alho H, Wu X, Hervonen A, Toluanen JP. Control of arterial tone after long-term coenzyme Q<sub>10</sub> supplementation in senescent rats. *Br J Pharmacol* 1998;**124**:1500-1506.
10. Watts GF, Playford DA, Croft KD, Ward NC, Mori TA, Burke V. Coenzyme Q(10) improves endothelial dysfunction of the brachial artery in Type II diabetes mellitus. *Diabetologia* 2002;**45**:420-426.
11. Belardinelli R, Mucaj A, Lacalaprice F, Solenghi M, Seddaiu G, Principi F, Tiano L, Littarru GP. Coenzyme Q<sub>10</sub> and exercise training in chronic heart failure. *Eur Heart J* 2006;**27**:2675-2681.
12. Hare JM, Stamler JS. NO/redox disequilibrium in the failing heart and cardiovascular system. *J Clin Invest* 2005;**115**:509-517.
13. Karlsson K, Marklund SL. Heparin-induced release of extracellular superoxide dismutase to human blood plasma. *Biochem J* 1987;**242**:55-59.
14. Oyanagui Y. Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Anal Biochem* 1984;**142**:290-296.
15. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 1969;**244**:6049-6055.
16. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Derhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. A Report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;**39**:257-265.
17. Clarkson P, Montgomery HE, Mullen MJ, Donald AE, Powe AJ, Bull T, Jubb M, World M, Deanfield JE. Exercise training enhances endothelial function in young men. *J Am Coll Cardiol* 1999;**33**:1379-1385.
18. Estornell E, Fato R, Castelluccio C *et al.* Saturation kinetics of coenzyme Q in NADH and succinate oxidation in beef heart mitochondria. *FEBS Lett* 1992;**311**:107-109.
19. Rosenfeldt F, Marasco S, Lyon W *et al.* Coenzyme Q<sub>10</sub> therapy before cardiac surgery improves mitochondrial function and *in vitro* contractility of myocardial tissue. *J Thorac Cardiovasc Surg* 2005;**129**:25-32.
20. Marriage BJ, Clandinin MT, MacDonald IM *et al.* Cofactor treatment improves ATP synthetic capacity in patients with oxidative phosphorylation disorders. *Mol Genet Metab* 2004;**81**:263-272.
21. Amadio A, Palermo R, Pelsoni G, Littarru GP. Effect of CoQ<sub>10</sub> administration on VO<sub>2</sub> max and diastolic function in athletes. In: Folkers K, Littarru GP, Yamagami T, eds. *Biomedical and Clinical Aspects of Coenzyme Q*. Vol. VI. Elsevier North Holland; 1991.
22. Barbiroli B, Iotti S, Lodi R. Improved brain and muscle mitochondrial respiration with CoQ. An *in vivo* study by 31P-MR spectroscopy in patients with mitochondrial cytopathies. *Biofactors* 1999;**9**:253-260.
23. Fukai T, Folz RJ, Landmesser U, Harrison GD. Extracellular superoxide dismutase and cardiovascular disease. *Cardiovasc Res* 2002;**55**:239-249.
24. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000;**101**:1899-1906.
25. Langsjoen P, Langsjoen A. Overview of the use of CoQ<sub>10</sub> in cardiovascular disease. *BioFactors* 1999;**9**:273-284.
26. Littarru GP, Lippa S, De Sole P, Oradei A. *In vitro* effect of different ubiquinones on the scavenging of biologically generated O<sub>2</sub><sup>-</sup>. *Drugs Exp Clin Res* 1985;**XI**:529-532.
27. Petrucci R, Giorgini E, Damiani E, Carloni P, Marrosu G, Trazza A, Littarru GP, Greci L. A study on the interactions between Coenzyme Q<sub>10</sub> and superoxide anion. Could ubiquinones mimic superoxide dismutase (SOD)? *Res Chem Intermed* 2000;**26**:269-282.
28. Groneberg DA, Kindermann B, Althammer M, Klapper M, Vormann J, Littarru GP, Doring F. Coenzyme Q<sub>10</sub> affects expression of genes involved in cell signalling, metabolism and transport in human CaCo-2 cells. *Int J Biochem Cell Biol* 2005;**37**:1208-1218.
29. Belardinelli R, Georgiou D, Purcaro A. Low dose dobutamine echocardiography predicts improvement in functional capacity after exercise training in patients with ischemic cardiomyopathy: prognostic implication. *J Am Coll Cardiol* 1998;**31**:1027-1034.
30. Clarkson P, Montgomery HE, Mullen MJ, Donald AE, Powe AJ, Bull T, Jubb M, World M, Deanfield JE. Exercise training enhances endothelial function in young men. *J Am Coll Cardiol* 1999;**33**:1379-1385.
31. Matsuo S, Matsumoto T, Takashima H, Ohira N, Yamane T, Yasuda Y, Tarutani Y, Horie M. The relationship between flow-mediated brachial artery vasodilation and coronary vasomotor responses to bradykinin: comparison with those to acetylcholine. *J Cardiovasc Pharmacol* 2004;**44**:164-170.