Cardiovascular mortality and N-terminal-proBNP reduced after combined selenium and coenzyme Q10 supplementation: A 5-year prospective randomized double-blind placebo-controlled trial among elderly Swedish citizens☆

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1. Introduction

Selenium is an essential nutrient required for vital processes within the body such as antioxidant defense, oxidative metabolism, and immune surveillance [1,2]. Dietary selenium is assimilated into selenoproteins, of which 25 are currently known in humans [1]. These include glutathione peroxidase (Gpx), thioredoxin reductase 1 (TrxR1), selenoprotein P, and iodothyronine deiodinases [3]. Dietary supplementation of selenium induces a changed inflammatory response as shown by Goldson et al. [4]. There is a close connection between the selenium content of soil and selenium dietary intake, best exemplified by Keshan disease, an endemic cardiomyopathy found in selenium-deficient areas of inland China [5,6]. The daily intake of this nutrient is regarded as insufficient in many Western European countries and a dietary supplementation of selenium has been suggested [1]. Clark et al. have proposed that selenium affects tumor development [7]. The association between ischemic heart disease and selenium has been reported in several studies [8–10]. Salonen et al. observed a 2.9-fold increased risk of cardiovascular death in patients with low selenium levels [11]. However, the efficacy of selenium supplementation as a single dietary additive has been debated [8–10,12]. Absence of clinical effects may, in some cases, be explained by short-term intervention periods, coenzyme Q10 deficiency and/or low selenoprotein activity due to concomitant deficiency of isopentenyl Sec-tRNA, a factor necessary for efficient selenoprotein synthesis [13].

Coenzyme Q10 (also termed ubiquinone) is present in all cells of the body and has a central role as an electron carrier in the mitochondrial respiratory chain and in oxidative phosphorylation. Extra-
mitochondrial coenzyme Q10 is also an efficient lipid soluble antioxidant, protecting against lipid peroxidation. For a normal heart function a steady supply of coenzyme Q10 via the circulatory system or through endogenous synthesis is required. Endogenous synthesis of coenzyme Q10 in the body declines with age indicating a rational for supplementation in the elderly [14]. Already 40 years ago it was reported that 75% of ischemic heart disease patients exhibited low levels of coenzyme Q10 in the plasma and decreasing myocardial levels as the heart disease progressed [12,14].

Low myocardial levels of coenzyme Q10 have been observed in patients with cardiomyopathy [15,16]. Furthermore, non-surviving heart failure patients had lower levels of coenzyme Q10 in the plasma than surviving patients [17]. Dietary supplementation of coenzyme Q10 has been shown to improve the myocardial function and quality of life in patients with ischemic cardiomyopathy [18–20]. The cardio-protective effects of coenzyme Q10 are most likely explained by its antioxidant effect, which requires continuous reduction of ubiquinone and regeneration to the active ubiquinol form. Regeneration of ubiquinol requires selenium in the form of the selenoprotein TrxR1, which contains the unique amino acid selenocysteine (SeC) in its active site. In addition, the synthesis of SeC-containing proteins requires a functional mevalonate pathway, in which coenzyme Q10 is a product [13].

The aim of the present study was to evaluate whether combined supplementation of selenium and coenzyme Q10 in a primary health care cohort would affect the severity of chronic heart failure, all-cause mortality, and cardiovascular mortality. The rationale of this study is underlined by the fact that over 600 references (Pub Med) regarding Q10 and heart disease and over 800 references regarding selenium and heart disease in PubMed. However, most of these references are hypothesis generating basic science indicating the need for a clinical trial.

The secondary objective was to determine whether the intervention could influence cardiac function as evaluated by cardiac natriuretic peptides and echocardiography.

2. Methods

2.1. Study design

The present study was a prospective randomized double-blind placebo-controlled trial. A rural municipality of 10,300 inhabitants in south-east Sweden was selected for this intervention study. All citizens aged between 70 and 88 years (n = 1320) had previously participated in an epidemiological study and had been continuously followed with medical examinations since 1998. A total of 876 people accepted the invitation to that study. We invited these participants to participate in the present study. In 2003, at the start of this study, however, only 675 of the 876 participants were still alive and not seriously diseased. A total of 443 of the 675 accepted participation in this study which involved taking dietary supplements, and the follow-up program. The first participant was included in January 2003, and the last participant concluded this study in February 2010. All participants were examined by one of three experienced cardiologists. A new clinical history was recorded, a clinical examination was performed, the New York Heart Association functional class (NYHA class) was assessed, and an ECG and Doppler-echocardiographical examination were carried out. Blood pressure was measured with the participant resting in supine position. All participants were supplemented for 48 months, and were re-examined at the end of each six-month period. All-cause and cardiovascular mortalities were registered.

Thus 221 persons were given the active supplement of selenium + coenzyme Q10 (active treatment group), and 222 persons received the placebo supplement (placebo group). The design of the study is illustrated in Fig. 1. Informed consent was obtained from each patient. The study was approved by the Regional Ethical Committee and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The Medical Product Agency declined renewal of the study protocol since the study was not considered a trial of a medication for a certain disease but rather one of food supplement commodities that are commercially available. The study was registered at clinicaltrials.gov. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [35].

2.2. Blood samples

Blood samples were collected while the participants were resting in a supine position. Pre-chilled, EDTA-vials were used. The vials were centrifuged at 3000 \( \text{g, +4 °C,} \) and were then frozen at −70 °C. No sample was thawed more than twice.

**Fig. 1. Flow diagram of the study indicating the two groups; active treatment and placebo.**

- 443 persons accepted participation
- 221 randomized to active treatment group
- 222 randomized to placebo group
- 97 participants withdrew 0 lost during follow-up
- 118 participants withdrew 0 lost during follow-up
- 124 participants to analyze
- 104 participants to analyze

2.3. NT-proBNP

ProBNP, amino acids 1–76 (NT-proBNP), was measured on the Elecsys 2010 platform (Roche Diagnostics, Mannheim, Germany). The total coefficient of variation was 4.8% at 220 ng/L and 2.1% at 4254 ng/L (n = 70).

2.4. Echocardiography

Doppler echocardiographical examinations were performed with the participant in the left lateral position. The ejection fraction (EF) readings were categorized into four classes with interclass limits placed at 30%, 40% and 50% [22,23]. Normal systolic function was defined as EF ≥ 50%, while severely impaired systolic function was defined as EF < 30%.

2.5. Study intervention

All participants were randomized in blocks of 6 in a double-blind manner and given either a combination of 200 mg/day of coenzyme Q10 capsules (Bio-Quinon 100 mg, BLD, Pharma Nord, Vejle, Denmark) and 200 μg/day of organic selenium yeast tablets (SelenoPrecise 200 μg, Pharma Nord, Vejle, Denmark), or similar placebo. The study supplementation was taken in addition to regular medication. All study medications (active drug and placebo) not consumed were returned and counted.

The selenium source was a patented selenium yeast, SelenoPrecise®, of a pharmaceutical quality and has a documented batch-to-batch stability in its composition of selenium species [24–26]. Previous results from the Precise pilot studies showed low levels of adverse effects and good absorption [25] in doses up to 300 μg/day. It has been approved as a pharmaceutical drug in Denmark by the Danish Medicines Agency for many years (appr. no. 6233603).

The coenzyme Q10 preparations have shown good absorption and efficacy in previous controlled trials [27,28], and the capsules were identical to medicinal quality capsules registered for heart failure in a European Union Member State (Myoquin®, authorization no. GCV1 11494-2010).

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The patients with the following conditions were excluded from the study.

Recent myocardial infarction (within 4 weeks).

Planned cardiovascular operative procedure within 4 weeks.

Hesitation concerning whether the candidate can decide for him/herself to participate in the study or not, or if he/she understands the consequences of participation.

Serious disease that substantially reduces survival or when it was not expected that the participant could cooperate for the full 4 year period.

Other factors making participation unreasonable, such as long/complicated transport to the Primary Health Center where the project was managed, or drug/alcohol abuse.

2.7. Statistical methods

Descriptive data are presented as percentages or mean±SD. Student’s unpaired t-test was used for continuous variables and the Chi-square test was used for discrete variables. Cox proportional hazard regression analysis and Kaplan–Meier analysis were used to assess the risk of mortality during the follow-up period, and the odds ratio of the influence of active treatment on changes in NT-proBNP plasma concentration levels was calculated using logistic regression.

Echocardiographical evaluations were used to identify the EF of the participants at the start of the study and at end of the study. The EF results were grouped into four EF classes used routinely in cardiology; >50%, 40–50%, 30–40%, and <30%. Changes of the EF class were then scored +1 if improved, 0 if unchanged, and −1 if impaired. The statistical significance of the difference in EF change score between the two treatment groups was evaluated using a chi-square test for trend.

A P-value of <0.05 was considered statistically significant. All data were analyzed using a standard software (Statistica v. 10.0, Statsoft Inc, Tulsa, OK).

2.8. Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report. The corresponding author had full access to all the data in the study and had the final responsibility in the decision to submit the report for publication.

3. Results

3.1. Study population

The demographics of the two study groups (active treatment and placebo) showed equal gender distribution (Table 1). In Table 1 both the initial study population and the study population at the end of the study are presented, with regard to active treatment and placebo. The placebo group contained a higher number of participants who were being treated with ACE-inhibitors. Apart from this, the two groups were balanced at the start of the study. A number of participants (n=215) decided to discontinue the study: 43.9% in the active treatment group versus 53.2% in the placebo group (P=0.03). The two study populations at the study end were not significantly different apart from the changes in NT-proBNP concentration, and the corresponding changes in cardiac function changes as recorded in those who accepted an echocardiography at the end of the study (Table 1). The reasons for discontinuation of the study are

Table 2

<table>
<thead>
<tr>
<th>Reason for drop-out</th>
<th>Active substance n (%)</th>
<th>Placebo n (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too many tablets</td>
<td>21 (9.5)</td>
<td>37 (16.7)</td>
<td>P=0.03</td>
</tr>
<tr>
<td>Unknown reason</td>
<td>19 (8.6)</td>
<td>27 (12.2)</td>
<td>P=0.20</td>
</tr>
<tr>
<td>Chosen to discontinue the study</td>
<td>21 (9.5)</td>
<td>26 (11.7)</td>
<td>P=0.45</td>
</tr>
<tr>
<td>Malignant disease</td>
<td>2 (1.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other diseases</td>
<td>20 (9.0)</td>
<td>13 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal symptoms/diarrhea</td>
<td>9 (4.1)</td>
<td>7 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Not possible to draw blood samples</td>
<td>3 (1.4)</td>
<td>2 (1.0)</td>
<td></td>
</tr>
</tbody>
</table>

Note: the participants had been informed that according to the Helsinki Declaration they were not forced to give any reason for discontinuation of the study.

Table 1

<table>
<thead>
<tr>
<th>Initial population</th>
<th>Final population at study end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active n (%)</td>
<td>Placebo n (%)</td>
</tr>
<tr>
<td>Age years mean (SD)</td>
<td>78.0 (3.2)</td>
</tr>
<tr>
<td>Males/females n</td>
<td>115/106</td>
</tr>
<tr>
<td>History</td>
<td></td>
</tr>
<tr>
<td>Smokers (present) n (%)</td>
<td>21 (9.5)</td>
</tr>
<tr>
<td>Diabetes n (%)</td>
<td>47 (21.3)</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>158 (71.5)</td>
</tr>
<tr>
<td>Previous or present tumor n (%)</td>
<td>33 (14.9)</td>
</tr>
<tr>
<td>Previous thrombosis n (%)</td>
<td>52 (23.5)</td>
</tr>
<tr>
<td>IHD n (%)</td>
<td>47 (21.3)</td>
</tr>
<tr>
<td>NYHA class I n (%)</td>
<td>118 (53.4)</td>
</tr>
<tr>
<td>NYHA class II n (%)</td>
<td>61 (27.6)</td>
</tr>
<tr>
<td>NYHA class III n (%)</td>
<td>41 (18.5)</td>
</tr>
<tr>
<td>NYHA class IV n (%)</td>
<td>0</td>
</tr>
<tr>
<td>Unclassified NYHA n (%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: ACE: ACE-inhibitors; ARB: angiotensin receptor blockers; EF: ejection fraction; IHD: ischemic heart disease; IQR: inter quartile range; NT-proBNP: N-terminal fragment of proBNP; NYHA: New York Heart Association functional class; SD: standard deviation.
presented in Table 2. We conclude that an analysis of reasons for withdrawals in the active and placebo groups did not show any significant differences. It should be emphasized that all participants were informed that according to the Helsinki Declaration they did not have to give any reason for discontinuation of the study. The only adverse effect reported by the participants was symptoms of diarrhea (7/221 in the active group and 4/222 in the placebo group), which we believe were caused by the vehicle oil of the Q10 capsules. No other adverse effects were identified during the intervention period.

The median follow-up time of the total study population was 1891 days (range 348–1900 days), whereas the median follow-up time of the non-survivors was 1162 days (range 348–1900 days). Among the survivors a median follow-up time of 1864 days (range 1704–1900 days) was noted.

3.2. Intention to treat analysis

The intention to treat analysis uses data from all participants, including those who did not complete the trial.

3.2.1. Cardiovascular mortality

Cardiovascular mortality was 28/222 (12.6%) in the placebo group and 13/221 (5.9%) in the active treatment group with a significant difference in the mortality (χ²: 5.97; P = 0.015). Kaplan–Meier survival curves for the participants on active treatment vs. those on placebo are shown in Fig. 2. A significant risk reduction of cardiovascular mortality was found (HR, 0.45 [95% CI 0.24–0.89]; P = 0.02) in the univariate analysis. The significant effect of the active supplement was also evident in the multivariate analysis when analyzed together with other well-known factors that influence cardiovascular risk (Table 3). The model was adjusted for age, gender, heredity, hypertension, diabetes, ischemic heart disease, smoking, NYHA class III, EF < 40%, Hb < 120 g/L, previous thrombosis, ACE-inhibitor treatment and beta blocker treatment.

3.2.2. All-cause mortality

During the follow-up period, 36 participants (16.2%) in the placebo group suffered all-cause mortality compared with 28 participants (12.7%) in the active treatment group (χ²: 1.1; P = 0.29). In a univariate hazard proportional regression analysis, a hazard ratio of 0.76 (95% CI 0.47–1.26) with a P-value of 0.29 was found. Thus no significant difference in all-cause mortality was noted between the active treatment and placebo groups.

3.3. Biochemical analyses and echocardiography

The cardiac natriuretic peptide NT-proBNP was analyzed in the study population plasma samples according to routine. Participants’ NT-proBNP plasma concentration levels were measured at study onset, 24 months, and 48 months (Table 4). A significant difference in NT-proBNP plasma concentration levels between the two groups was noted at 24 months (P = 0.048), and this was further pronounced at 48 months (P = 0.014).

Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>All-cause mortality</th>
<th>Cardiovascular mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Male gender</td>
<td>2.11</td>
<td>1.21–3.70</td>
</tr>
<tr>
<td>Age</td>
<td>1.08</td>
<td>1.00–1.17</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.39</td>
<td>0.75–2.59</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.31</td>
<td>0.73–2.34</td>
</tr>
<tr>
<td>IHD</td>
<td>0.85</td>
<td>0.45–1.62</td>
</tr>
<tr>
<td>Smoker</td>
<td>1.37</td>
<td>0.68–2.76</td>
</tr>
<tr>
<td>NYHA class III</td>
<td>1.88</td>
<td>1.06–3.33</td>
</tr>
<tr>
<td>EF&lt;40%</td>
<td>1.81</td>
<td>0.82–4.00</td>
</tr>
<tr>
<td>Hb&lt;120 g/L</td>
<td>1.10</td>
<td>0.52–2.31</td>
</tr>
<tr>
<td>Prev thrombosis</td>
<td>1.30</td>
<td>0.75–2.27</td>
</tr>
<tr>
<td>ACEI</td>
<td>0.78</td>
<td>0.41–1.47</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>0.80</td>
<td>0.44–1.42</td>
</tr>
<tr>
<td>Active treatment</td>
<td>0.78</td>
<td>0.47–1.28</td>
</tr>
</tbody>
</table>

Note: ACEI: ACE-inhibitors; CI: confidence interval; EF: ejection fraction; IHD: ischemic heart disease; NYHA: New York Heart Association functional class.
The cardiac systolic function was evaluated by echocardiography. A reduced number of participants could be examined with echocardiography at the study start, compared to the number of echocardiographies at the study end. Therefore an analysis of the two populations (active supplementation vs. placebo) was performed at the start. No significant difference between the two populations could be seen. However during the study period significantly more participants did not attend the final echocardiography in the placebo group compared to the active supplementation group (102 participants out of 221 in the active supplementation group vs. 128 participants out of 222 in the placebo group; $\chi^2 = 0.02$). An evaluation of those that did drop-out during the intervention period shows that there were a significantly higher drop-out number the lower EF the participant had at the start of the study ($P = 0.03$). Finally, there was a tendency to a higher drop-out number in the placebo group at the start of the study among those with at least moderately impaired EF compared to the active supplementation group ($P = 0.077$). Thus, it could be stated that the placebo group was a significantly different group regarding cardiac function as described by echocardiography compared to the active supplementation group at the end of the study. More severely diseased participants have either died, or dropped-out during the intervention period in this group.

When analyzing the change of cardiac function according to echocardiography a significantly better cardiac function score could be found in the active supplementation group compared to the placebo group ($\chi^2 = 4.57; P = 0.03$).

3.4. Per protocol analysis

A per protocol analysis of cardiovascular and all-cause mortality has also been performed. As the number of participants who died due to cardiovascular related mortality (2 out of 124 on active supplementation, and 6 out of 104 on placebo), and regarding all-cause mortality (3 out of 124 on active supplementation and 7 out of 104 on placebo) was small, we did not perform any further statistical analyses.

4. Discussion

The rational of the present study conducted in an elderly Swedish population with dietary supplementation of selenium and coenzyme Q10 for 48 months was based on observations that the intake of these micronutrients is sub-optimal, and that there is a key intracellular relationship between the two substances which has previously not been evaluated in long-term, population based trials [5]. We hypothesized that in order to be efficient, a selenium supplementation should be combined with coenzyme Q10, as shown by Xia et al. [29]. Furthermore, we decided to provide the participants with fairly high doses of the combined substances, and preparations with documented bioavailability were chosen for the study.

In a previous small study of 61 patients with acute myocardial infarction in 1994, Kukliniski et al. showed a tendency of myocardial damage reduction and lower cardiovascular mortality in a treatment group. The participants were given a single dose of selenium, administered as an i.v. infusion, and thereafter a combination of selenium and coenzyme Q10 for the following year [30].

In the presented study, the participants in the active treatment group had a significantly reduced risk of cardiovascular mortality compared with participants in the placebo group as analyzed in the univariate Cox proportional hazard regression analysis. The survival curves (Kaplan–Meier analysis) support this effect on cardiovascular mortality after the long-term selenium and coenzyme Q10 supplementation and risk assessment in a multivariate setting showed a hazard ratio almost identical to that in the univariate analysis, (0.46; 95% CI 0.24–0.90, $P = 0.02$).

The significantly higher values of natriuretic peptide NT-proBNP in the placebo group support the positive effects of the supplements seen in the cardiac performance of participants in the active selenium/coenzyme Q10 treatment group, which were noted both at 24 and 48 months after the treatment started.

The positive influence on the cardiomycocytes is also illustrated by an increased EF, which was more common among those on active treatment. It is interesting to note that even in this small study population, and even if the two groups at the end of the intervention were not comparable as the most diseased participants have already disappeared from the placebo group, it is still a significantly more positive development of cardiac function as seen on the echocardiography in the active supplementation group compared to the placebo group.

Thus, two different methods showed improved myocardial performance after treatment in addition to a significant decrease in cardiovascular mortality. Recently Fumagalli et al. reported increased tolerance to exercise in a heart failure population when treated with Q10 plus creatinine compared to those on a placebo, indicating a positive effect on the cardiomycocytes comparable to the results of this study [31].

Several risk factors for cardiovascular mortality are included in the analysis and it is therefore of interest that the combination of selenium and coenzyme Q10 supplementation shows a highly significant risk reduction. The mechanistic explanations for the study outcome may be multiple. Lubos et al. demonstrated an inverse relationship between selenium and cardiovascular mortality [32], and similar results have also been reported in an animal model [33].

We suggest a mechanism based on novel biochemical data that most likely explains the effect of the combined selenium–coenzyme Q10 supplementation. Selenium is essential for the optimal activity of selenoproteins, including TrxR1, a protein which is crucial for the efficacy of coenzyme Q10. Selenium supplementation is especially important in regions with low soil contents of selenium such as Scandinavia, where selenium content in food is not sufficient for saturation of selenoproteins with the consequently suboptimal activity of TrxR1. In fact, in one of our previous studies on heart failure patients, we reported on low Trx1 levels that did not match the increased thioredoxin/oxidative stress levels [34]. Extramitochondrial coenzyme Q10 is also active in several metabolic pathways in the myocardium including the antioxidant pathways, as discussed by de Longeir and Salen [21]. Individuals with suboptimal levels of either coenzyme Q10, selenium, or both, will therefore be at a higher risk of experiencing cardiovascular events.

5. Limitations

The limitations of this investigator initiated study are the number of participants ($n=443$), the restricted age span of the study population.
Additional contributions

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [35].

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