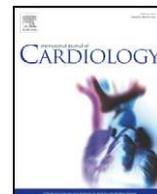




Contents lists available at SciVerse ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

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[☆]

Urban Alehagen^{a,d,*}, Peter Johansson^{a,d}, Mikael Björnstedt^b, Anders Rosén^c, Ulf Dahlström^{a,d}

^a Division of Cardiovascular Medicine, Department of Medicine and Health Sciences, Faculty of Health Sciences, Linköping University, Linköping, Sweden

^b Division of Pathology, Department of Laboratory Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden

^c Department of Clinical and Experimental Medicine, Division of Cell Biology, Linköping University, Linköping, Sweden

^d Department of Cardiology UHL, County Council of Östergötland, Linköping, Sweden

ARTICLE INFO

Article history:

Received 10 January 2012

Received in revised form 17 April 2012

Accepted 28 April 2012

Available online xxxx

Keywords:

Elderly

Selenium

Coenzyme Q10

Dietary supplementation

Cardiovascular mortality

NT-proBNP

ABSTRACT

Background: Selenium and coenzyme Q10 are essential for the cell. Low cardiac contents of selenium and coenzyme Q10 have been shown in patients with cardiomyopathy, but inconsistent results are published on the effect of supplementation of the two components separately. A vital relationship exists between the two substances to obtain optimal function of the cell. However, reports on combined supplements are lacking.

Methods: A 5-year prospective randomized double-blind placebo-controlled trial among Swedish citizens aged 70 to 88 was performed in 443 participants given combined supplementation of selenium and coenzyme Q10 or a placebo. Clinical examinations, echocardiography and biomarker measurements were performed. Participants were monitored every 6th month throughout the intervention.

The cardiac biomarker N-terminal proBNP (NT-proBNP) and echocardiographic changes were monitored and mortalities were registered. End-points of mortality were evaluated by Kaplan–Meier plots and Cox proportional hazard ratios were adjusted for potential confounding factors. Intention-to-treat and per-protocol analyses were applied.

Results: During a follow up time of 5.2 years a significant reduction of cardiovascular mortality was found in the active treatment group vs. the placebo group (5.9% vs. 12.6%; $P=0.015$). NT-proBNP levels were significantly lower in the active group compared with the placebo group (mean values: 214 ng/L vs. 302 ng/L at 48 months; $P=0.014$). In echocardiography a significant better cardiac function score was found in the active supplementation compared to the placebo group ($P=0.03$).

Conclusion: Long-term supplementation of selenium/coenzyme Q10 reduces cardiovascular mortality. The positive effects could also be seen in NT-proBNP levels and on echocardiography.

© 2012 Elsevier Ireland Ltd. All rights reserved.

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 S-adenosylmethionine, 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-

[☆] This study was registered at ClinicalTrials.gov, and has the identifier NCT01443780.

* Corresponding author at: Department of Medical and Health Sciences, Division of Cardiovascular Medicine, Heart Center, Linköping University, SE-581 85 Linköping, Sweden. Tel.: +46 10 103 0000.

E-mail address: Urban.Alehagen@liu.se (U. Alehagen).

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 rationale 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 the 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 review 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 g, +4 °C, and were then frozen at –70 °C. No sample was thawed more than twice.

Study flow diagram

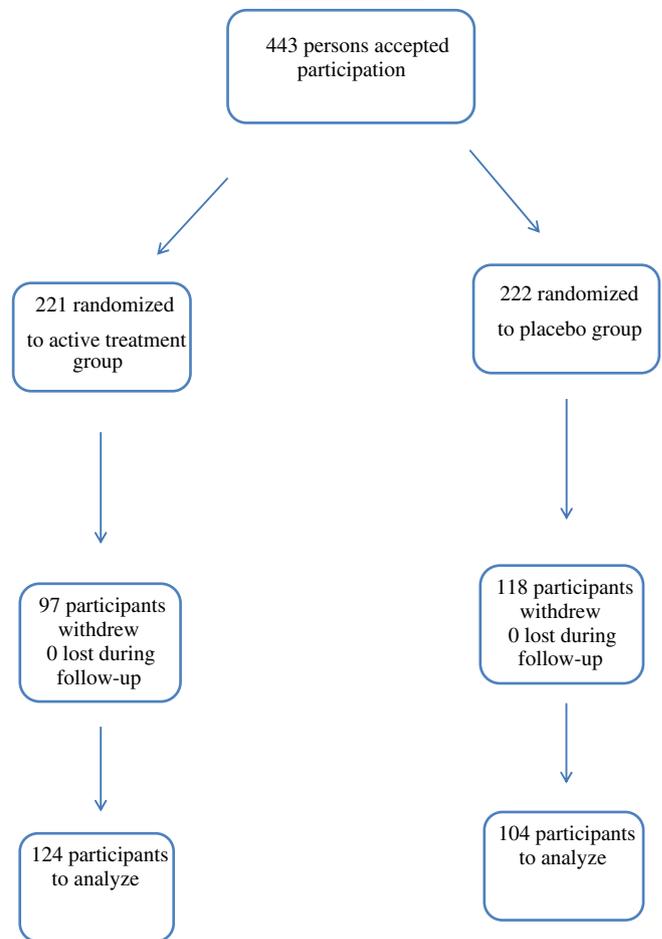


Fig. 1. Flow diagram of the study indicating the two groups; active treatment and placebo.

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 $EF \geq 50\%$, 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 B.I.D, 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 (Myoquinon®, authorization no. OGYI 11494-2010).

Table 1
Basal characteristics of the study population divided into active treatment and placebo.

	Initial population			Final population at study end		
	Active	Placebo	P-value	Active	Placebo	P-value
n	221	222		124	104	
Age years mean (SD)	78.0 (3.2)	78.2 (3.0)	0.36	76.2 (3.08)	76.2 (3.14)	
Males/females n	115/106	110/112		61/63	45/59	
<i>History</i>						
Smokers (present) n (%)	21 (9.5)	20 (9.0)	0.86	9 (7.3)	9 (8.7)	0.70
Diabetes n (%)	47 (21.3)	48 (21.6)	0.95	21 (16.9)	18 (17.3)	0.94
Hypertension n (%)	158 (71.5)	168 (75.7)	0.28	86 (69.4)	75 (72.1)	0.65
Previous or present tumor n (%)	33 (14.9)	38 (17.1)	0.83	17 (13.7)	15 (14.4)	0.88
Previous thrombosis n (%)	52 (23.5)	50 (22.5)	0.80	23 (18.5)	24 (23.1)	0.40
IHD n (%)	47 (21.3)	53 (23.9)	0.51	22 (17.7)	18 (17.3)	0.93
NYHA class I n (%)	118 (53.4)	108 (48.6)	0.32	75 (60.5)	61 (58.7)	0.78
NYHA class II n (%)	61 (27.6)	64 (28.8)	0.77	30 (24.2)	30 (28.8)	0.43
NYHA class III n (%)	41 (18.5)	47 (21.2)	0.49	19 (15.3)	12 (11.6)	0.41
NYHA class IV n (%)	0	0		0	0	
Unclassified NYHA n (%)	0	3 (1.4)		0	1 (1.0)	
<i>Medications</i>						
ACEI n (%)	35 (15.8)	54 (24.3)	0.03	17 (13.7)	14 (13.5)	0.96
ARB n (%)	10 (4.5)	13 (5.9)	0.53	4 (3.2)	7 (6.7)	0.22
Betablockers n (%)	81 (36.7)	73 (32.9)	0.40	45 (36.3)	34 (32.7)	0.57
Digitalis n (%)	11 (5.0)	11 (5.0)		6 (4.8)	1 (1.0)	0.09
Diuretics n (%)	70 (31.7)	88 (39.6)	0.08	40 (32.2)	35 (33.7)	0.82
Statins n (%)	45 (20.7)	51 (23.0)	0.50	27 (21.8)	18 (17.3)	0.40
<i>Examinations</i>						
EF<40% n (%)	16 (7.2)	17 (7.7)	0.87	7 (5.6)	4 (3.8)	0.53
NT-proBNP ng/L mean (IQR)	547 (399)	522 (308)		290 (199)	343 (246)	

Note: ACEI: 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.

2.6. Exclusion criteria

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 \pm SD. Student's unpaired two-sided *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

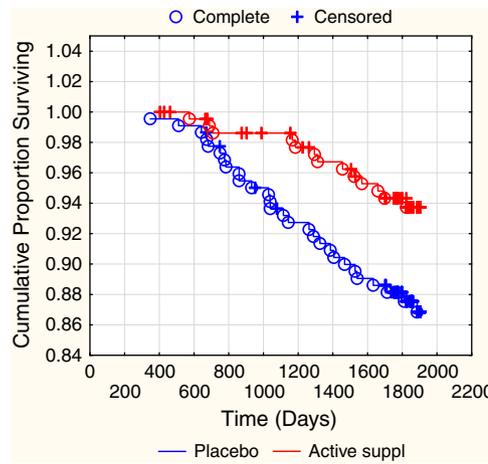
3.1. Study population

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 supplementation group versus 53.2% in the placebo group (χ^2 : 3.80; $P = 0.05$). 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
Reasons for drop-out from the intervention study with selenium and Q10 combined.

Reason for drop-out	Active substance n (%)	Placebo n (%)	P-value
Too many tablets	21 (9.5)	37 (16.7)	$P = 0.03$
Unknown reason	19 (8.6)	27 (12.2)	$P = 0.20$
Chosen to discontinue the study	21 (9.5)	26 (11.7)	$P = 0.45$
Malignant disease	2 (1.0)	0	
Other diseases	20 (9.0)	13 (5.9)	$P = 0.20$
Gastrointestinal symptoms/diarrhea	9 (4.1)	7 (3.2)	$P = 0.60$
Not possible to draw blood samples	3 (1.4)	2 (1.0)	$P = 0.65$

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.



Participants exposed

	Intention to treat						
	Start	300d	600d	900d	1200d	1500d	1800d
Active suppl	221	221	217	212	207	202	156
Placebo	222	222	220	210	202	196	152

Fig. 2. A Kaplan–Meier analysis of cardiovascular mortality among individuals on active treatment of selenium plus coenzyme Q10 supplementation versus individuals on placebo treatment, in an elderly population during a follow-up period of 5.2 years. The analysis is according to the intention to treat principle. Note: Censored participants were participants who were still alive at the end of the study period or who had died of reasons other than cardiovascular disease. Completed participants comprised those who had died due to cardiovascular disease.

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.

Table 3

Multivariate Cox proportional hazard regression analysis of intervention with selenium + coenzyme Q10 in the study population according to the intention to treat principle and during 48 months of intervention.

Variable	All-cause mortality			Cardiovascular mortality		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Male gender	2.11	1.21–3.70	0.009	2.78	1.34–5.80	0.006
Age	1.08	1.00–1.17	0.05	1.13	1.03–1.26	0.01
Hypertension	1.39	0.75–2.59	0.29	1.18	0.56–2.52	0.66
Diabetes	1.31	0.73–2.34	0.36	1.09	0.51–2.34	0.83
IHD	0.85	0.45–1.62	0.63	0.78	0.35–1.73	0.54
Smoker	1.37	0.68–2.76	0.37	1.50	0.65–3.44	0.34
NYHA class III	1.88	1.06–3.33	0.03	2.06	1.01–4.19	0.05
EF<40%	1.81	0.82–4.00	0.14	1.40	0.50–3.94	0.53
Hb<120 g/L	1.10	0.52–2.31	0.80	1.24	0.52–2.96	0.63
Prev thrombosis	1.30	0.75–2.27	0.35	1.60	0.82–3.10	0.17
ACEI	0.78	0.41–1.47	0.44	0.84	0.39–1.84	0.67
Beta blockers	0.80	0.44–1.42	0.44	0.87	0.42–1.82	0.71
Active treatment	0.78	0.47–1.28	0.33	0.46	0.24–0.90	0.02

Note: ACEI: ACE-inhibitors; CI: confidence interval; EF: ejection fraction; IHD: ischemic heart disease; NYHA: New York Heart Association functional class.

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 (χ^2 : 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 (χ^2 : 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 4

Description of median plasma values of NT-proBNP (1st–4th quartiles) and the median percentage calculated change from baseline to 24 and 48 months in those with active treatment versus placebo.

Month	Active treatment	Placebo	P-value
	NT-proBNP ng/L median (1st–4th quartiles)	NT-proBNP ng/L median (1st–4th quartiles)	
Baseline	(n = 218) 215 (118–521)	(n = 222) 253 (141–481)	0.25
24	(n = 144) 200 (105–428)	(n = 124) 241.5 (166–509)	0.048
	Md change % from month 0 0.03 (–0.22–0.52)	Md change % from month 0 0.19 (–0.14–0.64)	0.14
48	(n = 119) 215 (116–437)	(n = 97) 304 (168–644)	0.014
	Md change % from month 0 0.17 (–0.18–0.77)	Md change % from month 0 0.52 (0.07–1.02)	0.015

Note: Md: median; NT-proBNP: N-terminal fragment of proBNP.

The cardiac systolic function was evaluated by echocardiography. A reduced number of participants could be examined with echocardiography at the study end, compared to the number of echocardiographies at the study start. 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; χ^2 : 5.87; $P=0.02$). 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 significant better cardiac function score could be found in the active supplementation group compared to the placebo group (χ^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 rationale 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, Kuklinski 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 cardiomyocytes 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 cardiomyocytes 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 Lorgeril 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

(70–88 years), and the number of premature withdrawals from the study. The study is underpowered as a result of the limited size of the study population. However, we forward that as this is the first attempt to study the interrelated action of selenium and coenzyme Q10 first described by Xia et al. [29], it should be regarded as hypothesis generating, and renders further research. Results described in terms of reduction of cardiovascular mortality, results on cardiac function (echocardiography), and results displayed in reduced plasma concentration of NT-proBNP, all need to be re-examined in a larger study, and in a younger population in order to reduce the withdrawal because of impaired health among the participants.

The reason behind withdrawing from the study was known in 78% of cases (Table 4). The primary reason for withdrawal was the fact that the micronutrient supplements were given in addition to regular medication over a long period (48 months). Patient compliance is a recognized problem when multiple preparations are prescribed. Besides the number of preparations, illness from other diseases/malignant conditions were one of the known main reasons for withdrawal. However, the second most important reason for withdrawal from the study was the participants' age (median age > 78 years), reduced strength and long transportation time in this rural area during the relatively long intervention time.

Despite the limited size due to long intervention in an elderly population, this study is giving new and interesting hypothesis generating results.

6. Conclusion

Supplementation with selenium and coenzyme Q10 resulted in a significant reduction of cardiovascular mortality in a cohort of Swedish elderly persons all living in a rural municipality. The biomarker of cardiomyocyte wall tension, NT-proBNP, was significantly lowered in agreement with the mortality data. In addition, the echocardiographic measurements support a positive effect of the supplements. This study investigates for the first time the interrelationship between selenium and coenzyme Q10 and the compounds have been used in a therapeutic purpose to affect the cardiomyocyte. Generalization of the results requires further population-based studies designed to evaluate changes in mortality. We suggest that the results of this study can provide a basis for extended analyses of the effects of selenium/coenzyme Q10 on various conditions, and that further supplementation studies in larger populations involving diverse age groups should be initiated.

Author contributions

Dr Alehagen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Alehagen, Dahlström, Rosén, Björnstedt. *Acquisition of data:* Alehagen, Johansson. *Analysis and interpretation of data:* Alehagen. *Drafting of the manuscript:* Alehagen. *Critical revision of the manuscript:* Alehagen, Dahlström, Rosén, Johansson, Björnstedt. *Statistical analysis:* Alehagen. *Obtained funding:* Alehagen. *Study supervision:* Alehagen, Johansson.

Conflict of interest disclosure

All authors have completed and submitted the OCMJE form for Disclosure and Potential Conflicts of Interest and none were reported.

Funding/support

Part of the analysis costs were supported by grants from Pharma Nord Aps, Denmark, the County Council of Östergötland, Linköping University, and the Swedish Research Council (AR). The selenium tablets and coenzyme Q10 capsules were kindly donated by Pharma Nord Aps.

Additional contributions

We would like to thank Drs. Claes Post, Jens Rehfeld, and P-G Larsson for their expert revision of the manuscript. No economic compensation was rewarded.

Acknowledgement

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

References

- [1] Rayman MP. The importance of selenium to human health. *Lancet* 2000;356(9225):233–41.
- [2] Selenius M, Rundlöf AK, Olm E, Fernandes AP, Björnstedt M. Selenium and the selenoprotein thioredoxin reductase in the prevention, treatment and diagnostics of cancer. *Antioxid Redox Signal* 2010;12(7):867–80.
- [3] Fairweather-Tait SJ, Bao Y, Broadley MR, et al. Selenium in human health and disease. *Antioxid Redox Signal* 2011;14(7):1337–83.
- [4] Goldson AJ, Fairweather-Tait SJ, Armah CN, et al. Effects of selenium supplementation on selenoprotein gene expression and response to influenza vaccine challenge: a randomised controlled trial. *PLoS One* 2011;6(3):e14771.
- [5] Rayman MP. Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proc Nutr Soc* 2005;64(4):527–42.
- [6] Cheng YY, Qian PC. The effect of selenium-fortified table salt in the prevention of Keshan disease on a population of 1.05 million. *Biomed Environ Sci* 1990;3(4):422–8.
- [7] Clark LC, Combs Jr GF, Turnbull BW, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA* 1996;276(24):1957–63.
- [8] Bleyts J, Navas-Acien A, Laclaustra M, et al. Serum selenium and peripheral arterial disease: results from the national health and nutrition examination survey, 2003–2004. *Am J Epidemiol* 2009;169(8):996–1003.
- [9] Eaton CB, Abdul Baki AR, Waring ME, Roberts MB, Lu B. The association of low selenium and renal insufficiency with coronary heart disease and all-cause mortality: NHANES III follow-up study. *Atherosclerosis* 2010;212(2):689–94.
- [10] Stranges S, Marshall JR, Trevisan M, et al. Effects of selenium supplementation on cardiovascular disease incidence and mortality: secondary analyses in a randomized clinical trial. *Am J Epidemiol* 2006;163(8):694–9.
- [11] Salonen JT, Alfthan G, Huttunen JK, Pikkariainen J, Puska P. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. *Lancet* 1982;2(8291):175–9.
- [12] Venardos KM, Perkins A, Headrick J, Kaye DM. Myocardial ischemia–reperfusion injury, antioxidant enzyme systems, and selenium: a review. *Curr Med Chem* 2007;14(14):1539–49.
- [13] Moosmann B, Behl C. Selenoproteins, cholesterol-lowering drugs, and the consequences: revisiting of the mevalonate pathway. *Trends Cardiovasc Med* 2004;14(7):273–81.
- [14] Kalén A, Appelkvist EL, Dallner G. Age-related changes in the lipid compositions of rat and human tissues. *Lipids* 1989;24(7):579–84.
- [15] Folkers K, Vadhanavikit S, Mortensen SA. Biochemical rationale and myocardial tissue data on the effective therapy of cardiomyopathy with coenzyme Q10. *Proc Natl Acad Sci U S A* 1985;82(3):901–4.
- [16] Senes M, Erbay AR, Yilmaz FM, et al. Coenzyme Q10 and high-sensitivity C-reactive protein in ischemic and idiopathic dilated cardiomyopathy. *Clin Chem Lab Med* 2008;46(3):382–6.
- [17] Molyneux SL, Florkowski CM, George PM, et al. Coenzyme Q10: an independent predictor of mortality in chronic heart failure. *J Am Coll Cardiol* 2008;52(18):1435–41.
- [18] Witte KK, Nikitin NP, Parker AC, et al. The effect of micronutrient supplementation on quality-of-life and left ventricular function in elderly patients with chronic heart failure. *Eur Heart J* 2005;26(21):2238–44.
- [19] Sander S, Coleman CI, Patel AA, Klüger J, White CM. The impact of coenzyme Q10 on systolic function in patients with chronic heart failure. *J Card Fail* 2006;12(6):464–72.
- [20] Mortensen SA. Coenzyme Q10 as an adjunctive therapy in patients with congestive heart failure. *J Am Coll Cardiol* 2000;36(1):304–5.
- [21] de Lorgeril M, Salen P. Selenium and antioxidant defenses as major mediators in the development of chronic heart failure. *Heart Fail Rev* 2006;11(1):13–7.
- [22] Jensen-Urstad K, Bouvier F, Hojer J, et al. Comparison of different echocardiographic methods with radionuclide imaging for measuring left ventricular ejection fraction during acute myocardial infarction treated by thrombolytic therapy. *Am J Cardiol* 1998;81(5):538–44.
- [23] van Royen N, Jaffe CC, Krumholz HM, et al. Comparison and reproducibility of visual echocardiographic and quantitative radionuclide left ventricular ejection fractions. *Am J Cardiol* 1996;77(10):843–50.
- [24] Larsen EH, Sloth JJ, Hansen M, Moesgaard S. Selenium speciation and isotope composition in 77Se-enriched yeast using gradient elution HPLC separation and ICP-dynamic reaction cell-MS. *J Anal At Spectrom* 2003;18:310–6.
- [25] Larsen EH, Hansen M, Paulin H, Moesgaard S, Reid M, Rayman M. Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies. *J AOAC Int* 2004;87(1):225–32.

- [26] Bugel S, Larsen EH, Sloth JJ, et al. Absorption, excretion, and retention of selenium from a high selenium yeast in men with a high intake of selenium. *Food Nutr Res* 2008;52.
- [27] Weis M, Mortensen SA, Rassing MR, Moller-Sonnergaard J, Poulsen G, Rasmussen SN. Bioavailability of four oral coenzyme Q10 formulations in healthy volunteers. *Mol Aspects Med* 1994;15:s273–80 [Suppl.].
- [28] Folkers K, Moesgaard S, Morita M. A one year bioavailability study of coenzyme Q10 with 3 months withdrawal period. *Mol Aspects Med* 1994;15:s281–5 [Suppl.].
- [29] Xia L, Nordman T, Olsson JM, et al. The mammalian cytosolic selenoenzyme thioredoxin reductase reduces ubiquinone. A novel mechanism for defense against oxidative stress. *J Biol Chem* 2003;278(4):2141–6.
- [30] Kuklinski B, Weissenbacher E, Fahnrich A. Coenzyme Q10 and antioxidants in acute myocardial infarction. *Mol Aspects Med* 1994;15:s143–7 [Suppl.].
- [31] Fumagalli S, Fattiroli F, Guarducci L, et al. Coenzyme Q10 terclatrate and creatine in chronic heart failure: a randomized, placebo-controlled, double-blind study. *Clin Cardiol* 2011;34(4):211–7.
- [32] Lubos E, Sinning CR, Schnabel RB, et al. Serum selenium and prognosis in cardiovascular disease: results from the AtheroGene study. *Atherosclerosis* 2010;209(1):271–7.
- [33] Lymbury RS, Marino MJ, Perkins AV. Effect of dietary selenium on the progression of heart failure in the ageing spontaneously hypertensive rat. *Mol Nutr Food Res* Oct 2010;54(10):1436–44.
- [34] Jekell A, Hossain A, Alehagen U, Dahlström U, Rosén A. Elevated circulating levels of thioredoxin and stress in chronic heart failure. *Eur J Heart Fail* 2004;6(7):883–90.
- [35] Coats AJ, Shewan LG. Statement on authorship and publishing ethics in the international journal of cardiology. *Int J Cardiol* 2011;153(3):239–40.