Free Radical Biology and Medicine 204 (2023) 207–214

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Selenium and coenzyme Q_{10} improve the systemic redox status while reducing cardiovascular mortality in elderly population-based individuals

Belinda J. Dunning [a], Arno R. Bourgonje [b,*], Marian L.C. Bulthuis [a], Jan Alexander [c], Jan O. Aaseth [d], Anders Larsson [e], Harry van Goor [a,1], Urban Alehagen [f,1]

[a] Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, the Netherlands
[b] Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, the Netherlands
[c] Norwegian Institute of Public Health, 0213, Oslo, Norway
[d] Department of Research, Innlandet Hospital Trust, 2381, Brumunddal, Norway
[e] Faculty of Health and Social Sciences, Inland Norway University of Applied Sciences, 2624, Lillehammer, Norway
[f] Division of Cardiovascular Medicine, Department of Medical and Health Sciences, Linköping University, 581 85, Linköping, Sweden

ARTICLE INFO

Keywords:
Selenium
Coenzyme Q_{10}
Thiols
Oxidative stress
Redox
Cardiovascular disease

ABSTRACT

Background: Serum sulfhydryl groups (R–SH, free thiols) reflect the systemic redox status in health and disease, and may be amenable to therapeutic modulation. Since R–SH are readily oxidized by reactive species, oxidative stress is characterized by reduced serum R–SH levels. Selenium and coenzyme Q_{10} supplementation may improve the systemic redox status. This study aimed to evaluate the effect of supplementation with selenium and coenzyme Q_{10} on serum free thiols and to study associations with the risk of cardiovascular mortality in elderly community-dwelling individuals.

Methods: In this randomized, double-blind, placebo-controlled trial, serum R–SH were measured colorimetrically and adjusted for albumin in 434 individuals at baseline and after 48 months of intervention. Selenium yeast (200 μg/day) and coenzyme Q_{10} (200 mg/day) or placebo were provided as dietary supplements.

Results: After 48 months of intervention, participants receiving combined selenium and coenzyme Q_{10} supplementation demonstrated increased levels of serum R–SH compared to placebo ($P = 0.002$). In prospective association analysis, the highest rate of cardiovascular mortality after a median follow-up of 10 years (IQR: 6.8–10.5) was observed in the lowest quartile (Q1) of R–SH levels. Baseline albumin-adjusted serum R–SH were significantly associated with the risk of cardiovascular mortality, even after adjustment for potential confounding factors (hazard ratio [HR] 1.98 per SD, 95% CI: 1.34–2.91, $P < 0.001$).

Conclusion: Supplementation with selenium and coenzyme Q_{10} to an elderly community-dwelling population low on the two substances, significantly improved serum R–SH levels, supporting a reduction in systemic oxidative stress. Low serum R–SH levels were significantly associated with an increased risk of cardiovascular mortality in elderly individuals.

1. Introduction

Cardiovascular diseases (CVD), e.g. ischemic heart disease (IHD), stroke, heart failure, and peripheral arterial disease, are major contributors to human disability and the leading cause of global mortality [1]. Over the past decades, the CVD burden has increased globally by 12.5% and 50.4% in terms of disability-adjusted life years and death, respectively [2–5]. Risk factors for developing CVDs include age and lifestyle factors such as smoking, an unhealthy diet and physical inactivity [6]. These factors, in turn, can result in hypertension, hypercholesterolemia, and decreased glucose tolerance. These phenomena drive the process of atherosclerosis and might induce heart damage, which may eventually result in cardiovascular death [7]. In addition, CVD poses a significant financial burden to global economies as CVD-related costs are increasing rapidly [8,9].

Selenium (Se) is a trace element that plays an essential role in normal

* Corresponding author. Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, 9713, GZ, Groningen, the Netherlands.
E-mail address: a.r.bourgonje@umcg.nl (A.R. Bourgonje).
[1] These authors contributed equally.

https://doi.org/10.1016/j.freeradbiomed.2023.04.024
Received 20 March 2023; Received in revised form 17 April 2023; Accepted 30 April 2023
Available online 11 May 2023

cellular functioning [10]. Selenium mediates its effects through incorporation as selenocysteine into selenoproteins. A daily intake of at least 100 μg/day of selenium is required to achieve optimal expression of selenoproteins in plasma [11]. However, selenium in the soil and consequently dietary intake vary greatly per region. In Europe, dietary selenium and human levels are generally lower than in individuals living in North America [12]. Therefore, supplementation or fortification has been suggested in European countries [13]. Importantly, low levels of selenium have been associated with an increased risk of mortality, impaired immune function, and neurodegenerative decline [14,15]. Enzymatic reactions of the selenoenzymes have priorly been linked to reductions in free thiols. An example of this is the selenoenzyme thioredoxin reductase which reduces thioredoxin, which in turn can reduce oxidized glutathione (GSSG) [16]. Coenzyme Q_{10} is endogenously produced and declines with age. It is important for cellular functioning, primarily working as a lipophilic antioxidant and crucial factor in the electron transport chain of mitochondria. Supplementary ubiquinone is the oxidized form of coenzyme Q_{10}. Ubiquinone is readily reduced to its active form ubiquinol in the body as a product of redox reactions [17,18]. Numerous enzyme systems facilitate the maintenance of adequate ubiquinol levels in the tissues [19]. Therefore, supplementation with ubiquinone increases the level of ubiquinol in the plasma and lipoproteins, thereby increasing the resistance to oxidation [20]. Additionally, coenzyme Q_{10} and selenium physiologically collaborate, as the selenoenzyme thioredoxin reductase is required to reduce the supplementary oxidized ubiquinone into its active form ubiquinol [21,22]. Cytosolic thioredoxin reductase-1 is inducible upon oxidative stress and other stresses, and for that to occur an adequate selenium status is needed [23,24].

In an elderly Swedish population low in selenium, selenium and coenzyme Q_{10} supplementation combined has been shown to reduce the risk of cardiovascular mortality up to 12 years. It also improved cardiac function and reduced levels of the natriuretic peptide NT-proBNP after five years [25–28]. Multiple selenoproteins are expressed in the heart and these play an important role in redox regulation and cardiac protection against oxidative stress [29]. Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of the former, leading to a disruption of redox signaling and control, and molecular damage caused by reactive oxygens species (ROS) [30,31]. Aside from ROS, sulfhydryl moieties are also receptive to oxidative modification by other types of reactive species, e.g. hydrogen sulfide (H_2S) and nitric oxide (NO)-related metabolites. Recently, an integrative conceptual framework, the reactive species interactome (RSI), was established that aims to describe the interactions among different types of reactive species, including ROS, reactive nitrogen species (RNS), reactive sulfur species (RSS), and reactive carbonyl species (RCS), as well as their interactions with downstream biological targets [31–33]. An important role in the RSI has been attributed to RSS, and cysteine-based redox switches (consisting of extracellular free thiols) play a pivotal role in the RSI since they serve as the main transducing components of redox regulation [31,34]. Our hypothesis is that protein free thiols in the extracellular compartment including plasma may act as central hubs of inter-organ redox communication, serving as multimodal redox relays by kinetically controlling intra- and extracellular exchange reactions, and forming a read-out of the global thiol redox status. Hence, systemic oxidative stress can be globally measured by circulating free thiols (FTs) in serum. Low molecular weight oxidized thiols expelled from the intracellular compartment may block plasma protein thiols by forming mixed disulfides, leading to a reduction in serum free thiols [44]. A reduced level of FTs may reflect systemic oxidative stress, since they are readily oxidized by ROS, providing a global reflection of the overall *in vivo* redox status [35]. Serum FTs have been shown to significantly predict the risk of CV-events and all-cause mortality [36]. Additionally, increased FT concentrations are associated with lower levels of NT-proBNP and favorable disease outcome [37].

In this study, we aimed to investigate the effect of selenium and coenzyme Q_{10} supplementation on systemic FT levels as a proxy of oxidative stress in an elderly population consisting of elderly Swedish citizens low in selenium. Secondly, we aimed to evaluate whether baseline levels of serum FTs are associated with CVD-associated mortality. Following these aims, we hypothesized that the protective effect of combined selenium and coenzyme Q_{10} supplementation in this population, if related to reduced systemic oxidative stress, would be reflected by increased levels of FTs, and that serum FTs may associate with the risk of CV-mortality in elderly individuals.

2. Materials and methods

2.1. Study design

The study was a prospective randomized double-blind placebo-controlled trial [26]. Participants were selected from a rural municipality in south-east Sweden for this intervention study. In 1996, citizens aged between 70 and 88 years (n = 1,320) living in this municipality were invited as part of an epidemiological program, of which 876 people accepted the invitation. In 2003, at the start of this study, 675 of the 876 participants were still alive and not seriously diseased. A total of 443 individuals finally accepted participation in the study, which involved daily supplement intake and a follow-up program. In total, 221 participants were randomized to the intervention group which involved dietary active supplementation of 100 mg BID of coenzyme Q_{10} capsules (Bio-Quinon 100 mg B.I.D, Pharma Nord, Vejle, Denmark) and 100 μg BID of organic selenium yeast tablets (SelenoPrecise 100 μg, Pharma Nord, Vejle, Denmark). In addition, 222 participants were randomized to receive placebo. The dietary supplementation was taken in addition to any regular medication. All participants were supplemented for 48 months. Participants were examined at inclusion and re-examined after each six-month period by one of three experienced cardiologists. This involved the recording of a new clinical history and the performance of a clinical examination, including blood pressure and assessment of the New York Heart Association functional class (NYHA class). An electrocardiogram and a Doppler-echocardiographic examination were performed at start and at the end of the intervention. Health related quality-of-life was also evaluated at start, after 24 months and at the end of the intervention. Blood samples were drawn at each 6 months interval during the intervention period. Written informed consent was obtained from all patients. The study was approved by the Regional Ethical Committee in Linköping, Sweden, and conforms to the ethical guidelines of the Declaration of Helsinki (2013).

2.2. Exclusion criteria

Exclusion criteria included myocardial infarction in the past 4 weeks; planned cardiovascular operative procedure within 4 weeks; uncertainty concerning the capacity of the candidate to decide for themselves to participate in the study or not, or whether the candidate understood the consequences of participation; presence of serious disease that substantially reduces chances of survival or when it was not expected that the participant could cooperate for the full 48-month period; and other factors making participation unreasonable, such as long/complicated transport to the Primary Health Center where the project was managed or drug/alcohol abuse.

2.3. Data collection and storage

Blood samples were collected while the participants were resting in supine position. Pre-chilled, EDTA-vials were used. The vials were centrifuged at 3000×g at 4 °C, and were then frozen at −70 °C. No sample was thawed more than twice. Previous pilot experiments showed that freezing and thawing did not affect free thiol stability. Blood pressure was measured with the participant resting in supine position.

2.4. Laboratory measurements

Serum free thiols (R–SH, sulfhydryl groups) was measured via thawing and four-fold dilution using 0.1 M Tris buffer (pH 8.2) of serum samples. Background absorption was measured at 412 nm, in combination with a reference measurement at 630 nm, using the Varioskan microplate reader (Thermo Scientific, Breda, the Netherlands). Following this, 20 μL 1.9 mM 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB, Ellman's Reagent, CAS number 69-78-3, Sigma-Aldrich Corporation, St. Louis, MO, USA) in 0.1 M phosphate buffer (pH 7.0) was added to the samples. Absorbances were remeasured after an incubation time of 20 min at room temperature. Parallel measurement of an L-cysteine (CAS number 52-90-4, Fluka Biochemika, Buchs, Switzerland) calibration curve (range from 15.6 to 1000 in 0.1 M Tris/10 mM EDTA (pH 8.2) established final concentrations of serum FTs. Both intra- and interassay coefficients of variation of the FT measurements were <10%. NT-proBNP was measured using an electrochemiluminiscence immunoassay (Elecsys 2010, Roche Diagnostics, Mannheim, Germany) [38]. The analytical range was 5–35.000 ng/L (0.6–4130 pmol/L). Total coefficient of variation was 4.8% at the level of 217 ng/L (26 pmol/L) (n = 70) and 2.1% at the level of 4261 ng/L (503 pmol/L) at our laboratory. All blood samples were stored at − 70 °C, and none had been thawed before this analysis. All samples were analyzed within two weeks of inclusion in the study.

2.5. Statistical analysis

Baseline demographic and clinical data were presented as proportions with corresponding percentages (%) or mean ± standard deviations (SD). Differences between groups were tested using unpaired two-sided t-tests for continuous variables and chi-square tests for discrete variables. Potential interactions of covariates in comparisons of FT concentrations between groups were tested with ANCOVA, and in all statistical evaluations of FT we adjusted for a potential effect of treatment allocation. All prognostic FT evaluations were performed adjusted for serum albumin levels by dividing baseline FT levels and serum albumin in order to indirectly account for total thiol content [31]. Survival distributions during the follow-up period were assessed for below- (Q1-2) and above- (Q3-4)-median albumin-adjusted serum free thiol concentrations using Kaplan-Meier survival analysis. For this, free thiols were divided into four quartiles (Q1: <56.9; Q2: 56.9–94.0; Q3: 94.0–121.1; Q4: >121.1). Repeated measures of variance were used in order to analyse individual changes in the concentration of the biomarker analyzed, compared to group mean values. Cox proportional hazards regression analyses were performed to assess the association between serum FT levels and cardiovascular mortality, expressed by hazard ratios (HRs) with corresponding 95% confidence intervals (CIs). Serum R–SH levels were expressed per SD increment or decrement (using Z-scores) to facilitate results interpretation. For each predictor, the proportionality of hazards assumption was checked to verify absence of violation. Multivariable Cox regression analyses were performed to adjust for relevant clinical confounders, including treatment allocation, followed by stratified analyses to assess HRs across relevant subgroups. Restricted cubic splines (RCS) were fitted using three knots to assess the potential presence of non-linearity of the association between serum R–SH level and the risk of cardiovascular mortality, as evaluated in Cox regression analysis. Non-linearity was evaluated using likelihood ratio tests, in which nested models were compared using linear or linear and cubic spline terms. Two-sided P-values were considered statistically significant. Data analysis was performed using Statistica v. 13.2 (Dell Inc, Tulsa, OK, USA). Data visualization was performed using RStudio (v.1.2.1335, RStudio, Boston, MA) and the *matplotlib* (v.3.4.1) and *seaborn* (v.0.11.1) packages embedded in the Python programming language (v.3.9.0, Python Software Foundation).

3. Results

3.1. Baseline cohort characteristics

Baseline demographic and clinical characteristics of the sub-study cohort are presented in Table 1. Baseline selenium and coenzyme Q_{10} status are presented in Table 2. In total, 434 participants were included in this post-hoc analysis, of which 218 subjects received selenium and coenzyme Q_{10} supplementation and 216 subjects placebo. Mean age across all study participants was 77 years, and males and females were almost equally represented (50.6% vs. 49.4%). The main reason for drop-out was required intake of too many tablets.

3.2. Serum free thiols and selenium and Q_{10} supplementation in elderly citizens

At inclusion, mean serum free thiols in the active vs. placebo groups were 91.4 μM/L and 94.1 μM/L, respectively. After 48 months of supplementation of selenium and Q_{10}, mean serum free thiols increased significantly to 100.7 μM/L, compared with a mean serum free thiol decrease to 81.4 μM/L in the placebo group (P = 0.002) (Fig. 1). At 48 months, serum free thiols were significantly inversely associated with NT-proBNP levels (r = −0.33, P = 0.001).

3.3. Serum free thiols at baseline and the risk of cardiovascular mortality in elderly citizens

After a median follow-up of 10.0 years (IQR: 6.8–10.5), 128 participants died because of a cardiovascular (CV) event (29.5%). The highest rate of CV-mortality occurred in the lowest quartile of free thiols at baseline (Q1) (n = 43, 40.6%). Kaplan-Meier survival analysis demonstrated statistically significant survival distributions for participants with below-median (Q1-2) albumin-adjusted free thiols and above-median (Q3-4) participants (P < 0.001, log-rank test) (Fig. 2A). Cox proportional hazards regression analysis with stepwise adjustment for

Table 1
Baseline demographic and clinical characteristics of the study cohort at inclusion, divided by those randomized into active supplementation of selenium and coenzyme Q_{10} and those into placebo.

	Active treatment	Placebo	*P*-value
	n = 218	n = 216	
Age (years)	77 (3.6)	77 (3.4)	
Sex (males/females), n	113/105	105/111	
Smoking, n (%)	20 (9.2)	20 (9.3)	0.96
Medical history			
Hypertension, n (%)	155 (71.1)	164 (75.9)	0.22
IHD, n (%)	47 (21.6)	50 (23.1)	0.67
Diabetes, n (%)	46 (21.1)	47 (21.8)	0.85
NYHA class I, n (%)	117 (53.7)	106 (49.1)	0.31
NYHA class II, n (%),	60 (27.5)	64 (29.6)	0.60
NYHA class III, n (%)	40 (18.3)	43 (19.9)	0.66
NYHA class IV, n (%)	0 (0.0)	0 (0.0)	
Unclassified, n (%)	1 (0.5)	3 (1.4)	
Medication use			
Beta blockers, n (%)	79 (36.2)	69 (31.9)	0.36
ACEI, n (%)	38 (17.4)	50 (23.1)	0.14
Diuretics, n (%)	67 (30.7)	85 (39.4)	0.07
Statins, n (%)	45 (20.6)	49 (22.7)	0.61
Examinations			
EF < 40%, n (%)	15 (6.9)	15 (6.9)	0.97
NT-proBNP, ng/ml, mean (SD)	546.2 (1585.2)	496.9 (850.9)	0.69

Data are presented as mean (SD) or proportions n with corresponding percentages (%). *P-values were obtained using unpaired two-sided t-tests for continuous variables or chi-square tests for discrete variables. Abbreviations: ACEI, angiotensin-converting enzyme inhibitors; EF, ejection fraction; IHD, ischemic heart disease; NYHA, New York Heart Association functional class; SD, standard deviation.

Table 2
Selenium and coenzyme Q_{10} status of the study cohort pre- and post-intervention divided by those randomized into active supplementation of selenium and coenzyme Q_{10} and those into placebo.

	Active treatment	Placebo	*P*-value
	$n = 218$	$n = 216$	
S-Selenium pre-intervention, µg/l, mean (SD)	66.5 (15.9)	67.4 (17.2)	0.92
S-selenium post-intervention, µg/l, mean (SD)	210.3 (59.3)	71.6 (25.0)	
Q_{10} pre-intervention, mg/l, mean (SD)	0.82 (0.31)	0.82 (0.34)	0.98
Q_{10} post-intervention, mg/l, mean (SD)	2.19 (1.35)	0.90 (0.36)	

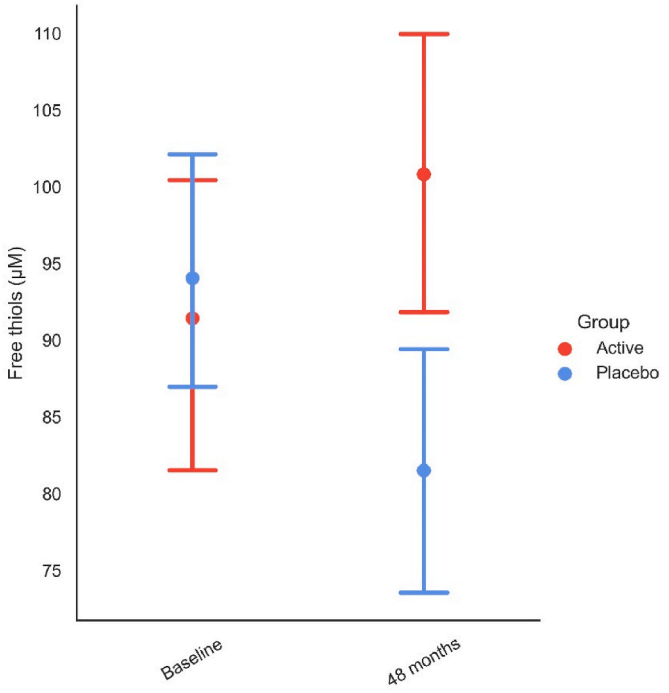

Fig. 1. Changes in serum free thiol levels after 48 months of intervention, consisting of either combined selenium and coenzyme Q_{10} supplementation (red) or placebo (blue). Changes were evaluated using repeated measures of variance methodology.

potential confounding factors showed statistically significant associations between albumin-adjusted serum free thiols and the risk of CV-mortality (Table 3, *Model 1*, hazard ratio [HR] per SD 2.02 [1.40–2.90], $P < 0.001$). After adjustment for treatment allocation, age, sex, the presence of hypertension, ischemic heart disease, diabetes, and atrial fibrillation, this association remained statistically significant (Table 3, *Model 3*, HR per SD 2.00 [1.37–2.93], $P < 0.001$). After additional adjustment for cardiac function (ejection fraction), hemoglobin and creatinine levels, and medication use, the association remained robust (Table 3, *Model 5*, HR per SD 1.98 [1.34–2.91], $P < 0.001$). Restricted cubic splines demonstrated no deviance from linear association between albumin-adjusted serum free thiols at baseline and the risk of CV-mortality ($P = 0.930$, likelihood ratio test) (Fig. 2B).

3.4. Stratified analyses

Stratified analyses for the association between albumin-adjusted serum free thiols at baseline and the risk of CV-mortality showed consistently increased risks in all analyzed subgroups (Fig. 3).

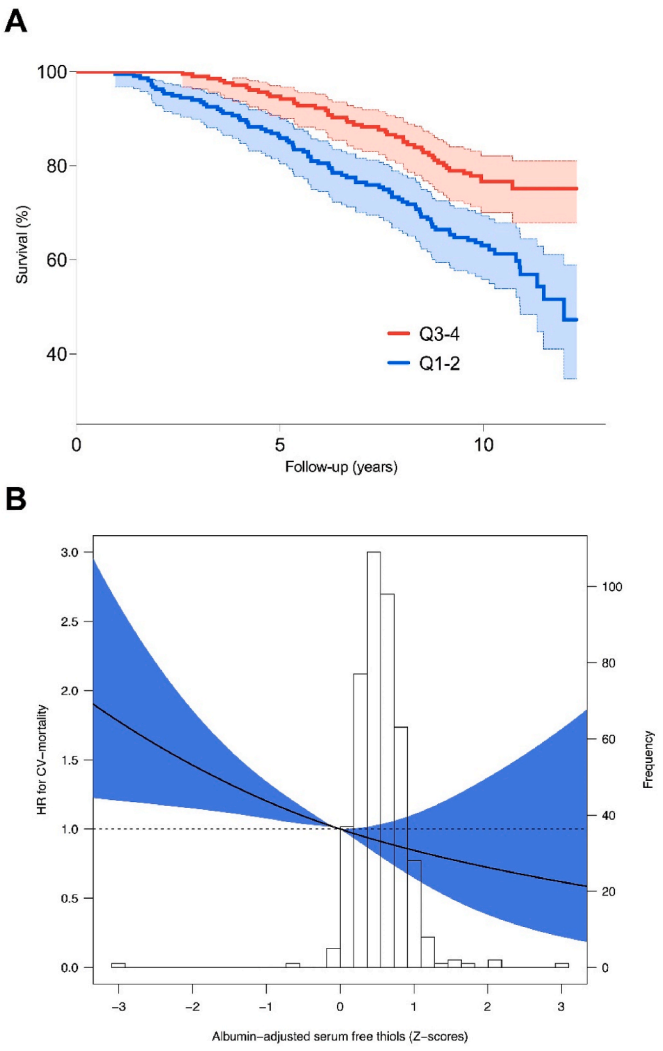

Fig. 2. (**A**) Kaplan-Meier survival curve for medians of albumin-adjusted serum free thiols at baseline, representing the CV-free survival across a median follow-up of 10 years. Lowest rates of survival were observed in individuals with below-median (Q1-2) albumin-adjusted serum free thiols compared to above-median free thiols (Q3-4). (**B**) Restricted cubic spline (RCS) demonstrating the association between albumin-adjusted serum free thiols and the risk for cardiovascular mortality. Estimated associations were derived from Cox proportional hazards regression models and RCS based on three knots (0.5th, 50th and 99.5th percentiles). A likelihood ratio test for nonlinearity was not significant. The blue-shaded area represents the 95% confidence interval.

Table 3
Cox proportional hazards regression analyses for associations between albumin-adjusted serum free thiols (below/above-median) and the risk of cardiovascular mortality.

	HR	95% CI	*P*-value
Model 1	2.02	1.40–2.90	<0.001
Model 2	1.83	1.26–2.67	0.002
Model 3	2.00	1.37–2.93	<0.001
Model 4	1.99	1.35–2.92	<0.001
Model 5	1.98	1.34–2.91	<0.001

Model 1, only adjusted for treatment allocation. Model 2, model 1 with adjustment for age and sex. Model 3, model 2 with adjustment for comorbidity including hypertension, ischemic heart disease, diabetes and atrial fibrillation. Model 4, model 3 plus additional adjustment for cardiac function (ejection fraction), hemoglobin and creatinine levels. Model 5, model 4 plus additional adjustment for medication use (beta blockers and ACE-inhibitors). Abbreviations: HR, hazard ratio; CI, confidence interval.

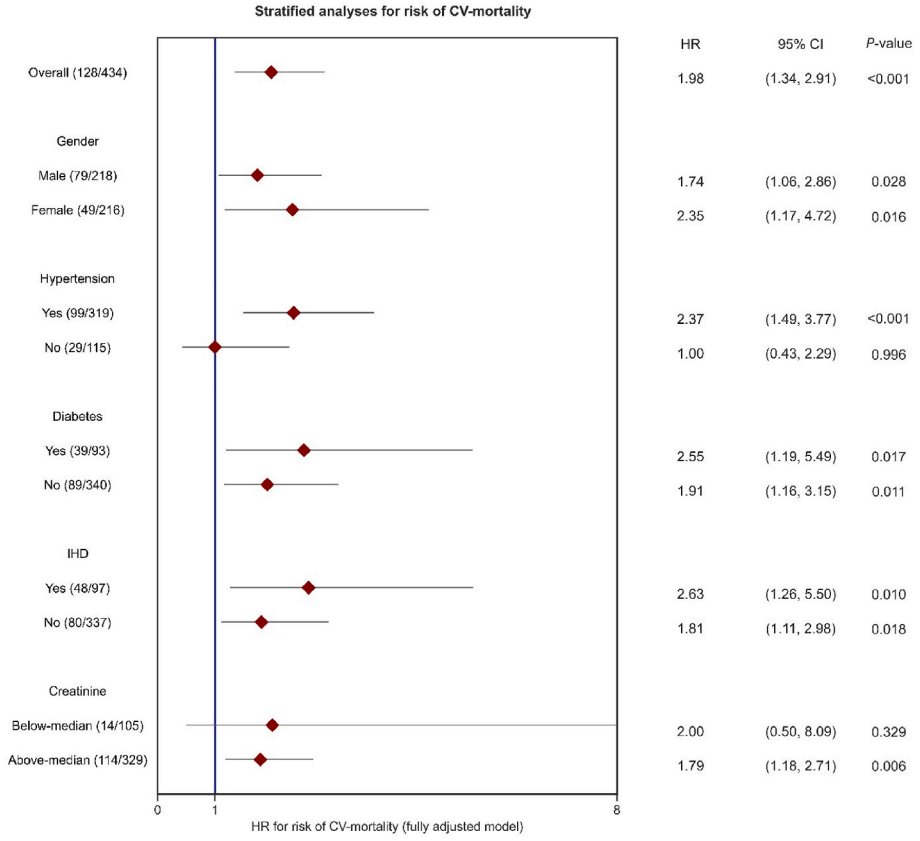

Fig. 3. Stratified Cox regression analyses for the association between albumin-adjusted serum free thiols at baseline and the risk of CV-mortality across various subgroups. Hazard ratios (HRs) are depicted with corresponding 95% confidence intervals (CI). HRs demonstrate consistently positive associations between albumin-adjusted serum free thiols and the risk of CV-mortality across all subgroups.

Corresponding HRs were relatively higher for females (2.35 (1.17–4.72)) compared to males (1.74 (1.06–2.86). Participants with existing comorbidities, including hypertension, diabetes, ischemic heart disease, as well as participants with above-median creatinine levels, demonstrated higher HRs for associations between albumin-adjusted serum free thiols and the risk of CV-mortality.

4. Discussion

This study demonstrated that combined selenium and coenzyme Q_{10} supplementation for 48 months significantly increased levels of albumin-adjusted serum free thiols in elderly individuals low in selenium, supporting a beneficial effect on systemic oxidative stress. Additionally, baseline albumin-adjusted serum free thiols were inversely associated with the risk of CV-mortality. After adjustment for traditional risk factors, the association between albumin-adjusted serum free thiols and risk for CV mortality remained statistically significant. As albumin is the dominant contributor to the extracellular free thiol pool (via its redox-active Cys^{34} residue) and as it is an important variable influencing CV prognosis, we have, when using baseline FT, consistently corrected all analyses for albumin [31]. That the supplementation of selenium and Q_{10} do influence the serum levels is shown from the measurements of the concentrations pre- and post-intervention (Table 2). Also, every 6 months at the visit when new tablets/capsules were dispensed, all non-consumed tablets were returned and counted. Therefore, it was possible to get information of how much was consumed of the supplementation. Collectively, the results point to the significance of active selenium and coenzyme Q_{10} supplementation for reducing the degree of systemic oxidative stress as reflected by serum FTs, and thereby potentially reducing the risk of CV mortality in those with a low intake of selenium. Our study further underscores the importance of oxidative stress in the risk of CV mortality and emphasizes the merit of selenium and coenzyme Q_{10} supplementation as a means to potentially reducing systemic oxidative stress.

Over the past couple of decades, several studies have suggested a pathophysiological role of oxidative stress in the onset and progression of CVD. Although the underlying etiology of CVD is multifactorial, oxidative stress has been shown to play a pivotal role in many cardiovascular processes. Oxidative stress has been defined as an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage [30]. ROS are involved in the regulation of cell proliferation, migration and death [39]. At the cardiovascular level, increased levels of ROS may reduce the bioavailability of nitric oxide (NO). Reduced NO availability, in turn, may lead to elevated vasoconstriction in the arteries, thereby contributing to the onset of hypertension [40,41]. Overproduction of ROS is also implicated in atherosclerotic plaque formation [42]. Additionally, ROS production has been linked to the development of heart failure [43]. Extracellular free thiols are in equilibrium with intracellular redox status, and may be blocked by intracellular-derived low molecular weight oxidized thiols, reducing the concentration of free thiols [44]. On the other hand, reduction in intracellular oxidative stress would result in an increase in free thiols in plasma. The quantification of free thiols enables to evaluate the systemic redox status and provides a representation of the degree of systemic oxidative stress *in vivo* [31]. Previously, thiols have been established as a useful biomarker in subjects with acute myocardial infarction [45]. It has been shown that plasma thiols are significantly lower in subjects with severe coronary artery disease [46]. Additionally, serum thiols are independently associated with CV risk scores at the population level [47]. In the present study, we tested the hypothesis whether serum free thiols would be associated with the risk of CV-mortality in elderly individuals, and indeed found

that serum free thiol concentrations (divided in Q1-Q4) at baseline were inversely associated with the risk of CV mortality. These findings are in line with previous study showing that serum free thiols form a predictor of CV-events and all-cause mortality in the general population [36].

Selenium plays an important role in cardiovascular health, as it exerts antioxidant effects through selenoproteins with antioxidative effects, by inhibiting inflammation and apoptosis, thereby preventing cardiovascular damage [48]. Additionally, selenium deficiency has previously been linked to multiple CVDs, including cardiomyopathies [49], atrial fibrillation [50], atherosclerosis [51], and heart failure [52]. Prior research on the effects of selenium on oxidative stress biomarkers accords well with our findings in that supplementation with selenium significantly increased total antioxidant capacity in those low on selenium [53]. This may be due to inducible selenoenzymes, in particular glutathione peroxidase-1 and cytosolic thioredoxin reductase-1, that carry strong antioxidant activity, thereby protecting against oxidative damage [54,55]. In mice, selenium-deficient diets are associated with reduced free thiol levels [56]. The thioredoxin system and its lipophilic partner CoQ_{10} are central in cellular redox regulation [21]. In the current study, our findings show that the protective effect of combined selenium and coenzyme Q_{10} supplementation after 48 months in elderly individuals is associated with increased levels of free thiols, reflecting a reduction in systemic oxidative stress. Furthermore, supplementation of coenzyme Q_{10} may have favorably affected mitochondrial bioenergetics and -function, thereby increasing the efficiency of cellular energy metabolism. In contrast, the placebo group showed decreased levels of circulating free thiols, reflecting increased systemic oxidative stress, as can be expected in an ageing population [57].

Strengths of the study include the size of the study population and the study design. Another major strength of this study is the well-optimized method of measuring serum free thiol concentrations, which provides reliable results of free thiols in the micromolar ranges. However, several limitations also warrant recognition. For example, considering that all participants were of Caucasian descent, the generalizability of our results in subjects with different ethnicities remains unknown. Similarly, the current study population has a restricted age-span (70–88 years); therefore, the generalizability of our results in a younger population remains to be elucidated. Also, as the population is living in an area where there are low levels of selenium in the soil, the obtained results are particularly relevant to elderly living in similar areas where there are low levels of selenium in the soil. Finally, the present study did only use one biomarker for oxidative stress: serum free thiols. Although causality has not unequivocally been established, our proposed mechanism is substantiated by the use of free thiols. This biomarker previously has been shown to give a robust and powerful reflection of the systemic *in vivo* redox status [31]. In support, from this population, results on the effects on copeptin and MR-proadrenomedullin, as indirect markers for oxidative stress, showed attenuated levels, indicating less oxidative stress as a result of supplementation with selenium and Q_{10} [58,59]. Still, we do acknowledge that the dynamic nature of oxidative stress as pathophysiological mechanism would deserve a more integrative approach to characterization of the redox signaling network, since this would combine read-outs of different types of reactive species as well as multiple redox-regulated metabolic pathways. Such "redox metabolomics" approaches are being developed, but are accompanied by several technological and methodological challenges [60]. Emerging reports encourage the use of these approaches, providing frameworks of criteria that candidate redox biomarkers should fulfill in order to reliably assess the human redox system in clinical situations [32,33,61]. For instance, an attempt to disentangle the human redox signaling network may comprise a 'multi-omics' approach targeted at the critical elements of the RSI, consisting of i) nutritional compounds (e.g. organic compounds like amino acids, inorganic compounds like H_2S, and cofactors such as vitamins), ii) the transducing elements, consisting of cysteine-based redox switches e.g. the circulating pool of free thiols, and iii) the stable end products of the RSI, encompassing S-, N- and O-derived metabolites [31]. Alternatively, a mass spectrometry-based analysis of the extracellular thiol metabolome, consisting of the measurement of 12 distinct analytes, including total free thiols, could be performed in several human biofluids [62]. This could develop into novel diagnostic platforms for patient stratification and monitoring effects of redox-modulating therapeutics. Provided that such measurements would be performed within well-characterized clinical cohorts, routine measurements of blood parameters, and perhaps complemented by other key factors central to redox-regulated metabolic pathways and/or mitochondrial function, possibilities may ensue for expanding the granularity of the human redox architecture while also pinpointing to the key hubs of interactions among the distinct types of redox species.

In conclusion, we show that serum free thiol levels are significantly increased by active selenium and coenzyme Q_{10} supplementation. Furthermore, CV mortality is significantly higher in those with lower free thiol concentrations at baseline. Taken together, this study shows that active supplementation of selenium and coenzyme Q_{10} in elderly low in selenium has a positive effect on serum free thiols as proxy of systemic oxidative stress, while at the same time reducing the risk of mortality due to cardiovascular events. Future research has to solidify the association between selenium and coenzyme Q_{10} supplementation, their positive impact on serum free thiols and reduced CV-mortality. Furthermore, follow-up studies are needed to investigate the generalizability of these findings to younger and more ethnically diverse populations. Finally, future prospective research is required to elucidate the clinical relevance of selenium supplementation in a population low on selenium, and to investigate potential correlations between selenium supplementation, oxidative stress and other pathologies.

Funding

Part of the analysis cost was supported by grants from Pharma Nord Aps, Denmark, and the County Council of Östergötland, Linköping University. The funding organisations had no role in the design, management, analysis, or interpretation of the data, nor in the preparation, review or approval of the manuscript. No economic compensation was distributed.

Author contributions

UA and HvG were involved in conceptualization and study design. UA, AL, JOA, JA and HvG were responsible for funding acquisition and resources. UA, HAL, JOA, JA, HvG, BJD, ARB and MLCB collected study data. UA, BJD and ARB performed data analysis and data visualization. BJD, ARB and UA wrote the first draft of the manuscript. All authors contributed to results interpretation, critically reviewed the manuscript, contributed to manuscript revision, and read and approved the final version of the manuscript to be submitted for publication.

Ethical considerations

This study was reviewed and approved by the Regional Ethical Committee in Linköping, Sweden, and conforms to the ethical guidelines of the Declaration of Helsinki (2013). All participants provided written informed consent was obtained from all patients.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors would like to express their gratitude towards all study participants.

References

[1] World Health Organization, Health topics (cardiovascular diseases and cancer) (Internet). Available from: https://www.euro.who.int/en/health-topics. (Accessed 26 September 2022).

[2] H. Wang, M. Naghavi, C. Allen, R. Barber, Z. Bhutta, A. Carter, et al., Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015, Lancet 388 (10053) (2016) 1459–1544.

[3] T. Vos, S. Lim, C. Abbafati, K. Abbas, M. Abbasi, M. Abbasifard, et al., Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019, Lancet 396 (10258) (2020) 1204–1222.

[4] G. Roth, D. Abate, K. Abate, S. Abay, C. Abbafati, N. Abbasi, et al., Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017, Lancet 392 (10159) (2018) 1736–1788.

[5] A. Moran, M. Forouzanfar, G. Roth, G. Mensah, M. Ezzati, C. Murray, et al., Temporal trends in ischemic heart disease mortality in 21 world regions, 1980 to 2010, Circulation 129 (14) (2014) 1483–1492.

[6] S. Yusuf, P. Joseph, S. Rangarajan, S. Islam, A. Mente, P. Hystad, et al., Modifiable risk factors, cardiovascular disease, and mortality in 155 722 individuals from 21 high-income, middle-income, and low-income countries (PURE): a prospective cohort study, Lancet 395 (10226) (2020) 795–808.

[7] J. Scott, Pathophysiology and biochemistry of cardiovascular disease, Curr. Opin. Genet. Dev. 14 (3) (2004) 271–279.

[8] A. Foy, J. Mandrola, 1, in: Heavy Heart. Primary Care: Clinics in Office Practice, vol. 45, 2018, pp. 17–24.

[9] A. Gheorghe, U. Griffiths, A. Murphy, H. Legido-Quigley, P. Lamptey, P. Perel, The economic burden of cardiovascular disease and hypertension in low- and middle-income countries: a systematic review, BMC Publ. Health 18 (1) (2018).

[10] S. Fairweather-Tait, Y. Bao, M. Broadley, R. Collings, D. Ford, J. Hesketh, et al., Selenium in human health and disease, Antioxidants Redox Signal. 14 (7) (2011) 1337–1383.

[11] O. Brodin, J. Hackler, S. Misra, S. Wendt, Q. Sun, E. Laaf, et al., Selenoprotein P as biomarker of selenium status in clinical trials with therapeutic dosages of selenite, Nutrients 12 (4) (2020) 1067.

[12] R. Stoffaneller, N.L. Morse, A review of dietary selenium intake and selenium status in Europe and the Middle East, Nutrients 7 (3) (2015) 1494–1537.

[13] M. Rayman, Selenium and human health, Lancet 379 (9822) (2012) 1256–1268.

[14] G. Barchielli, A. Capperucci, D. Tanini, The role of selenium in pathologies: an updated review, Antioxidants 11 (2) (2022) 251.

[15] U. Alehagen, P. Johansson, M. Björnstedt, A. Rosén, C. Post, J. Aaseth, Relatively high mortality risk in elderly Swedish subjects with low selenium status, Eur. J. Clin. Nutr. 70 (1) (2016 Jan) 91–96.

[16] A. Holmgren, J. Lu, Thioredoxin and thioredoxin reductase: current research with special reference to human disease, Biochem. Biophys. Res. Commun. 396 (1) (2010) 120–124.

[17] G. López-Lluch, J. del Pozo-Cruz, A. Sánchez-Cuesta, A.B. Cortés-Rodríguez, P. Navas, Bioavailability of coenzyme Q10 supplements depends on carrier lipids and solubilization, Nutrition 57 (2019) 133–140.

[18] W.V. Judy, The instability of the lipid-soluble antioxidant ubiquinol: Part 2-dog studies, Integr. Med. 20 (5) (2021) 26–30.

[19] D. Mantle, A. Dybring, Bioavailability of coenzyme Q10: an overview of the absorption process and subsequent metabolism, Antioxidants 9 (5) (2020) 386.

[20] D. Mohr, V.W. Bowry, R. Stocker, Dietary supplementation with coenzyme Q10 results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low-density lipoprotein to the initiation of lipid peroxidation, Biochim. Biophys. Acta Lipids Lipid. Metabol. 1126 (3) (1992) 247–254.

[21] L. Xia, T. Nordman, J. Olsson, A. Damdimopoulos, L. Björkhem-Bergman, I. Nalvarte, et al., The mammalian cytosolic selenoenzyme thioredoxin reductase reduces ubiquinone, J. Biol. Chem. 278 (4) (2003) 2141–2146.

[22] A. Kromer, B. Moosmann, Statin-induced liver injury involves cross-talk between cholesterol and selenoprotein biosynthetic pathways, Mol. Pharmacol. 75 (6) (2009) 1421–1429.

[23] W.L. Trigona, I.K. Mullarky, Y. Cao, L.M. Sordillo, Thioredoxin reductase regulates the induction of haem oxygenase-1 expression in aortic endothelial cells, Biochem. J. 394 (Pt 1) (2006) 207–216.

[24] L. Pu, Q. Meng, S. Li, Y. Wang, B. Liu, TXNRD1 knockdown inhibits the proliferation of endothelial cells subjected to oscillatory shear stress via activation of the endothelial nitric oxide synthase/apoptosis pathway, Biochim. Biophys. Acta Mol. Cell Res. 1870 (4) (2023 Feb 7), 119436.

[25] U. Alehagen, P. Johansson, M. Björnstedt, A. Rosén, U. Dahlström, 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, Int. J. Cardiol. 167 (5) (2013) 1860–1866.

[26] U. Alehagen, J. Aaseth, P. Johansson, Reduced cardiovascular mortality 10 Years after supplementation with selenium and coenzyme Q10 for four years: follow-up results of a prospective randomized double-blind placebo-controlled trial in elderly citizens, PLoS One 10 (12) (2015), e0141641.

[27] U. Alehagen, J. Aaseth, J. Alexander, P. Johansson, Still reduced cardiovascular mortality 12 years after supplementation with selenium and coenzyme Q10 for four years: a validation of previous 10-year follow-up results of a prospective randomized double-blind placebo-controlled trial in elderly, PLoS One 13 (4) (2018), e0193120.

[28] P. Johansson, Ö. Dahlström, U. Dahlström, U. Alehagen, Effect of selenium and Q10 on the cardiac biomarker NT-proBNP, Scand. Cardiovasc. J. 47 (5) (2013) 281–288.

[29] A. Al-Mubarak, P. van der Meer, N. Bomer, Selenium, Selenoproteins, and Heart Failure: Current Knowledge and Future Perspective, Current Heart Failure Reports, 2021.

[30] H. Sies, Oxidative stress: a concept in redox biology and medicine, Redox Biol. 4 (2015) 180–183.

[31] M.M. Cortese-Krott, A. Koning, G.G.C. Kuhnle, P. Nagy, C. Bianco, A. Pasch, et al., The reactive species interactome: evolutionary emergence, biological significance, and opportunities for redox metabolomics and personalized medicine, Antioxidants Redox Signal. 27 (10) (2017) 684–712.

[32] E. Malard, S. Valable, M. Bernaudin, E. Pérès, L. Chatre, The reactive species interactome in the brain, Antioxidants Redox Signal. 35 (14) (2021) 1176–1206.

[33] A.R. Bourgonje, M. Feelisch, K.N. Faber, A. Pasch, G. Dijkstra, H. van Goor, Oxidative stress and redox-modulating therapeutics in inflammatory bowel disease, Trends Mol. Med. 26 (11) (2020) 1034–1046.

[34] K. Ono, T. Akaike, T. Sawa, Y. Kumagai, D.A. Wink, D.J. Tantillo, et al., Redox chemistry and chemical biology of H2S, hydropersulfides, and derived species: implications of their possible biological activity and utility, Free Radic. Biol. Med. 77 (2014) 82–94.

[35] A. Banne, A. Amiri, R. Pero, Reduced level of serum thiols in patients with a diagnosis of active disease, J. Anti Aging Med. 6 (4) (2003) 327–334.

[36] A.E. Abdulle, A.R. Bourgonje, L.M. Kieneker, A.M. Koning, S. la Bastide-van Gemert, M.L.C. Bulthuis, et al., Serum free thiols predict cardiovascular events and all-cause mortality in the general population: a prospective cohort study, BMC Med. 18 (1) (2020).

[37] A.M. Koning, W.C. Meijers, A. Pasch, H.G.D. Leuvenink, A.S. Frenay, M.M. Dekker, et al., Serum free thiols in chronic heart failure, Pharmacol. Res. 111 (2016) 452–458.

[38] A. Karl Am Borgya, E. Gallusser, K. Huber, W. Krueger, Rollinger, et al., Development of a novel, N-terminal-proBNP (NT-proBNP) assay with a low detection limit, Scand. J. Clin. Lab. Invest. Suppl. 230 (1999) 177–181.

[39] W. Wang, P.M. Kang, Oxidative stress and antioxidant treatments in cardiovascular diseases, Antioxidants 9 (12) (2020) 1292.

[40] R.M. Touyz, Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension, Hypertension 44 (3) (2004) 248–252.

[41] A.R. Bourgonje, M.F. Bourgonje, A. Post, S. la Bastide-van Gemert, L.M. Kieneker, M.L.C. Bulthuis, et al., Systemic oxidative stress associates with new-onset hypertension in the general population, Free Radic. Biol. Med. 187 (2022) 123–131.

[42] A.J. Kattoor, N.V. Pothineni, D. Palagiri, J.L. Mehta, Oxidative stress in atherosclerosis, Curr. Atherosclerosis Rep. 19 (11) (2017).

[43] A. van der Pol, W.H. van Gilst, A.A. Voors, P. van der Meer, Treating oxidative stress in heart failure: past, present and future, Eur. J. Heart Fail. 21 (4) (2018) 425–435.

[44] C.F. Mueller, J.D. Widder, J.S. McNally, L. McCann, D.P. Jones, D.G. Harrison, The role of the multidrug resistance protein-1 in modulation of endothelial cell oxidative stress, Circ. Res. 97 (7) (2005 Sep 30) 637–644.

[45] H. Kundi, I. Ates, E. Kiziltunc, M. Cetin, H. Cicekcioglu, S. Neselioglu, et al., A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis, Am. J. Emerg. Med. 33 (11) (2015) 1567–1571.

[46] B. Bilir, D.C. Akkoyun, M. Aydin, D. Ozkaramanli-Gur, H. Degirmenci, N. Albayrak, et al., Association of coronary artery disease severity and disulphide/native thiol ratio, Electron. J. General Med. 14 (2) (2017).

[47] A.A. Mangoni, A. Zinellu, C. Carru, J.R. Attia, M. McEvoy, Serum thiols and cardiovascular risk scores: a combined assessment of transsulfuration pathway components and substrate/product ratios, J. Transl. Med. 11 (1) (2013).

[48] A. Shalihat, A.N. Hasanah, Lesmana R. Mutakin, A. Budiman, D. Gozali, The role of selenium in cell survival and its correlation with protective effects against cardiovascular disease: a literature review, Biomed. Pharmacother. 134 (2021), 111125.

[49] Y. Shi, W. Yang, X. Tang, Q. Yan, X. Cai, F. Wu, Keshan disease: a potentially fatal endemic cardiomyopathy in remote mountains of China, Front. Pediatr. 9 (2021).

[50] A.A. Al-Mubarak, N. Grote Beverborg, V. Zwartkruis, C. Van Deutekom, R. T. Gansevoort, S.J.L. Bakker, et al., Selenium deficiency is associated with new-onset atrial fibrillation in PREVEND: a prospective general population cohort, Eur. J. Prevent. Cardiol. 29 (Supplement_1) (2022).

[51] H. Liu, H. Xu, K. Huang, Selenium in the prevention of atherosclerosis and its underlying mechanisms, Metallomics 9 (1) (2017) 21–37.

[52] A.A. Al-Mubarak, P. van der Meer, N. Bomer, Selenium, Selenoproteins, and Heart Failure: Current Knowledge and Future Perspective, Current Heart Failure Reports, 2021.

[53] M. Hasani, S. Djalalinia, M. Khazdooz, H. Asayesh, M. Zarei, A.M. Gorabi, et al., Effect of selenium supplementation on antioxidant markers: a systematic review and meta-analysis of randomized controlled trials, Hormones (Basel) 18 (4) (2019) 451–462.

[54] L.-O. Klotz, Kroncke Klaus-Dietrich, D.P. Buchczyk, H. Sies, Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress, J. Nutr. 133 (5) (2003).

[55] M. Valko, C.J. Rhodes, J. Moncol, M. Izakovic, M. Mazur, Free radicals, metals and antioxidants in oxidative stress-induced cancer, Chem. Biol. Interact. 160 (1) (2006) 1–40.

[56] F.K.W. Hoffmann, A.C. Hashimoto, L.A. Shafer, S. Dow, M.J. Berry, P.R. Hoffmann, Dietary selenium modulates activation and differentiation of CD4+ T cells in mice through a mechanism involving cellular free thiols, J. Nutr. 140 (6) (2010) 1155–1161.
[57] I. Liguori, G. Russo, F. Curcio, G. Bulli, L. Aran, D. Della-Morte, et al., Oxidative stress, aging, and diseases, Clin. Interv. Aging 13 (2018) 757–772.
[58] U. Alehagen, J. Aaseth, P. Johansson, Less increase of copeptin and MR-proADM due to intervention with selenium and coenzyme Q10 combined: results from a 4-year prospective randomized double-blind placebo-controlled trial among elderly Swedish citizens, Biofactors 41 (6) (2015) 443–452.
[59] U. Alehagen, P. Johansson, E. Svensson, J. Aaseth, J. Alexander, Improved cardiovascular health by supplementation with selenium and coenzyme Q10: applying structural equation modelling (SEM) to clinical outcomes and biomarkers to explore underlying mechanisms in a prospective randomized double-blind placebo-controlled intervention project in Sweden, Eur. J. Nutr. 61 (6) (2022) 3135–3148.
[60] J. Santolini, S.A. Wootton, A.A. Jackson, M. Feelisch, The Redox architecture of physiological function, Curr Opin Physiol 9 (2019) 34–47.
[61] M. Feelisch, M.M. Cortese-Krott, J. Santolini, S.A. Wootton, A.A. Jackson, Systems redox biology in health and disease, EXCLI J 21 (2022) 623–646.
[62] T.R. Sutton, M. Minnion, F. Barbarino, G. Koster, B.O. Fernandez, A.F. Cumpstey, et al., A robust and versatile mass spectrometry platform for comprehensive assessment of the thiol redox metabolome, Redox Biol. 16 (2018) 359–380.