Clinical studies

Supplemental selenium and coenzyme Q10 reduce glycation along with cardiovascular mortality in an elderly population with low selenium status – A four-year, prospective, randomised, double-blind placebo-controlled trial

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ABSTRACT

Background: A low intake of selenium has been shown to increase the risk of cardiovascular mortality, and supplementation of selenium and coenzyme Q10 influences this. The mechanism behind is unclear although effects on inflammation, oxidative stress and microRNA expression have been reported.

Fructosamine, a marker of long-term glycaemic control, is also a marker of increased risk of heart disease and death, even in non-diabetics.

Objective: To analyse the impact of selenium and coenzyme Q10 supplementation on the concentration of fructosamine. Also, the relation between pre-intervention serum selenium concentration and the effect on fructosamine of the intervention was studied.

Methods: Fructosamine plasma concentration was determined in 219 participants after six and 42 months of intervention with selenium yeast (200 µg/day) and coenzyme Q10 (200 mg/ day) (n = 118 of which 20 had diabetes at inclusion), or placebo (n = 101 of which 18 had diabetes at inclusion). Pre-intervention, the serum selenium levels were 67 µg/L (active treatment group: 66.6 µg/L; placebo group: 67.4 µg/L), corresponding to an estimated intake of 35 µg/day. Changes in concentrations of fructosamine following intervention were assessed by the use of T-tests, repeated measures of variance, and ANCOVA analyses.

Results: Post-intervention selenium concentrations were 210 µg/L in the active group and 72 µg/L in the placebo group. A lower concentration of fructosamine could be seen as a result of the intervention in the total population (P = 0.001) in both the males (P = 0.04) and in the females (P = 0.01) in the non-diabetic population (P = 0.002), and in both the younger (< 76 years) (P = 0.01) and the older (≥76 years) participants (P = 0.03). No difference could be demonstrated in fructosamine concentration in the diabetic patients, but the total sample was small (n = 38). In subjects with a low pre-intervention level of serum selenium the intervention gave a more pronounced decrease in fructosamine compared with those with a higher baseline selenium level.

Conclusion: A significantly lower concentration of fructosamine was observed in the elderly community-living participants supplemented with selenium and coenzyme Q10 for 42 months compared to those on the placebo. As oxidative mechanisms are involved in the glycation of proteins, less glycoxidation could be a result of the supplementation of selenium and coenzyme Q10, which could have contributed to lower cardiac mortality and less inflammation, as has earlier been reported.

This study was registered at Clinicaltrials.gov, and has the identifier NCT01443780.

1. Introduction

Fructosamine is a ketoamine formed by the glycation of serum proteins, mainly albumins [1]. It reflects the glycaemic control over the last two to four weeks, whereas HbA1c, another glycation product, reflects the last two- to three-month period. However, it has also been shown that fructosamine is strongly associated with microvascular complications [2], and that both in populations with diabetes, and in non-diabetic populations, a higher concentration of fructosamine was associated with coronary heart disease, stroke, heart failure and death [3]. In a study including more than 149,000 individuals, Malmström et al. found that those with a higher concentration of fructosamine had increased risk for myocardial infarction and death [4]. Zaccardi et al. demonstrated increased risk for type II diabetes in those with high fructosamine concentration in a 23-year longitudinal population study [5]. Browner et al., in a study of 9704 elderly women, reported increased risk for cardiovascular mortality in those with high fructosamine concentration [6].

Selenium is one of the essential trace elements found as selenoenzymes in living cells [7,8]. Among these, selenoprotein P, glutathione peroxidases, and thioredoxin reductase, all protecting against oxidative stress, might be the most important ones.

In European populations, low dietary selenium intake is the result of low selenium content in the soil, and biofortification has therefore been proposed [9,10]. This contrasts with the selenium status in the United States where the selenium content in the soil is generally high. The estimated serum selenium concentrations in US citizens are generally above 120 μg/L [11,12], whereas concentrations well below 90 μg/L are reported from European countries [13–17].

Xia et al. demonstrated an important interrelationship between selenium and coenzyme Q10 (ubiquinone) as the selenoenzyme thioredoxin reductase is required to obtain the active form of coenzyme Q10 (ubiquinol) [18]. The mevalonate cycle in the cell is, for optimal functioning, both dependent on an adequate supply of coenzyme Q10 and synthesis of selenoproteins., An insufficiency in selenium and reduced thioredoxin reductase activity could therefore result in less than optimal concentrations of active coenzyme Q10 (ubiquinol) in the cell. Coenzyme Q10 is a powerful antioxidant protecting against lipid peroxidation [19]. It has also been reported that ubiquinone reduces the inflammatory response [20]. The endogenous production of coenzyme Q10 decreases continually after the age of 20, and the myocardial production is reduced to half at the age of 80 [21]. Thus, elderly people living in areas with low selenium content in the soil and food may be at increased risk of heart disease and cardiovascular death due to a possible deficiency of both these compounds. As patients with diabetes are already at increased cardiovascular risk due to increased inflammation and impaired redox balance, it is possible that these participants would benefit even more by intervention with selenium and coenzyme Q10. Improved redox balance could be obtained by intervention with coenzyme Q10 [22].

Our research group have demonstrated higher cardiovascular mortality in a community population with low plasma selenium concentration [23]. The average serum selenium level pre-intervention was 67 μg/L, corresponding to an estimated suboptimal selenium intake of 35 μg/day.

Therefore, a dietary supplementation trial was performed with both selenium and coenzyme Q10, or placebo on 443 elderly Swedish community members. The trial was conducted from 2003 until 2010 [24]. The intervention time was four years, and the follow-up after 5.2 years showed significantly reduced cardiovascular mortality, and improved cardiac function as evaluated by echocardiography, and a reduced increase of the N-terminal fragment of proBNP (NT-proBNP), a cardiac peptide biomarker that increases during increased myocardial distension, but also as a result of increasing age. Significant effects on inflammation, on oxidative stress, on insulin-like growth factor (IGF) 1 [25] and also on microRNA [26] have been reported as a result of the intervention. It has been suggested that hyperglycaemia contributes to oxidative stress and inflammation, which in turn leads to micro- and macro-vascular damage and ultimately to diabetic complications such as cardiovascular disease [27]. As elevated fructosamine levels are associated with an increased cardiovascular risk, we hypothesised that intervention with selenium and coenzyme Q10 would be reflected in the fructosamine concentration as a marker of cardiovascular risk.

The aim of the present sub-study was to examine whether intervention with selenium and coenzyme Q10 also influenced the fructosamine levels, and we also considered potential impacts in subgroups defined by gender, the presence of ischaemic heart disease (IHD), diabetes or high age. The potential role of the pre-intervention concentration of serum selenium on the fructosamine levels was also evaluated.

2. Methods

2.1. Subjects

The presented data is a secondary analysis of a subgroup of 219 individuals from a prospective randomised double-blind placebo-controlled trial in an elderly community population of 443 individuals in the age range of 70–88 years that has been previously reported (Supplemental Fig. 1) [24,28]. The participants in the main study received the intervention for 48 months, and were re-examined every six months. At inclusion, new patient records were obtained, all participants went through a clinical examination, the New York Heart Association functional class (NYHA class) was assessed, and an ECG and Doppler echocardiographical examinations were performed with the participant in the left lateral position. The ejection fraction (EF) readings were categorised into four classes with interclass limits placed at 30%, 40% and 50% [29,30]. Normal systolic function was defined as EF ≥ 50 %, while severely impaired systolic function was defined as EF < 30 %.

Informed consent was obtained from each patient. In the main study, 221 individuals received active supplementation of 200 μg/day organic selenium (SelenoPrecise®, Pharma Nord, Denmark), plus 200 mg/day of coenzyme Q_{10} (Bio-Quinon®, Pharma Nord, Denmark), and 222 individuals received a placebo.

The present subgroup comprised 219 participants. The figures were
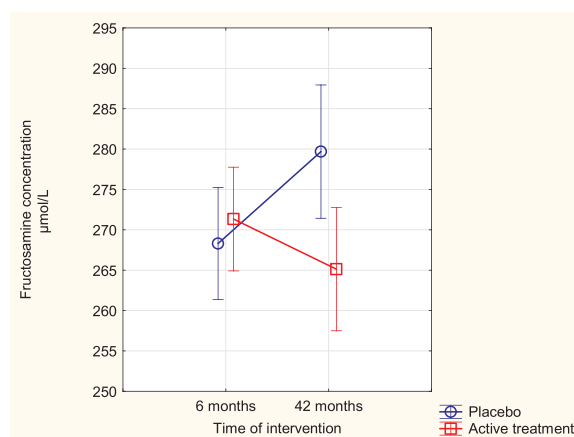
Fig. 1. Concentration of Fructosamine after 6 and 42 months in the selenium and coenzyme Q10 treatment group compared to the placebo group in the total study population.
Note: Evaluation performed by use of repeated measures of variance methodology
Note: Current effect: $F(1, 217) = 10.99$; $p = 0.001$
Note: Vertical bars denote 0.95 confidence intervals
Note: Blue circles: Placebo; Red squares: Active treatment group. Bars indicate ± 95 % CI

based on the number of participants still living and within the study, and providing blood samples after 42 months. The study was approved by the Regional Ethical Committee (No. D03−176; Forskningsetikkommmitten, Hälsouniversitetet, SE-581 85 Linköping, Sweden) and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. (The Medical Product Agency declined to review 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). This study was registered at Clinicaltrials.gov, and has the identifier NCT01443780, sept 30, 2011.

2.2. Biochemical analyses

Blood samples were collected after six and 42 months while the participants were resting in a supine position. Plasma prepared on pre-chilled, EDTA vials were used for analysis of fructosamine, while for analysis of selenium, full blood was collected on serum vials, which were centrifuged at 3000g, +4 °C, and then frozen at -70 °C. No sample was thawed more than once.

2.3. Evaluation of fructosamine

Fructosamine was analysed in plasma on a chemistry analyser (BS380, Mindray, Shenzhen, China) using reagents (04,537,939,190) from Roche Diagnostics (Rotkreuz, Switzerland). Precimat fructosamine (Ref 11098993, Roche Diagnostics) was used as a calibrator. The settings used were: endpoint method with blank reading at position 33–35 and final reading at position 56–59, wavelength (sub/main) 700/546, 10 μL sample volume, 150 μL R1 and 54 μL R2. The within-series CV was 0.73 % for control one (mean value 275 μmol/L) and 0.89 % for control two (mean value 515 μmol/L). The corresponding total CVs were 1.98 % and 1.65 %.

As regards the fructosamine analyses, an important issue to address is whether reliable results are obtained when using samples that have been stored at −70 °C for 42 months. In this respect, Nathan et al. reported an assay for glycated amines that demonstrated the stability of the samples also after up to 23 years of storage at −70 °C [31]. The stability of fructosamine during storage at −70 °C was also demonstrated by Koskinen and Irjala [32].

Another issue to address as regards fructosamine is the relationship between its plasma level and increasing age. Selvin et al. reported a slight increase in fructosamine level with increased age [33]. The same message was reported by Peng and Wei from a community-based population [34]. Chen et al. reported a significant increase in the levels of fructosamine as a function of age when producing reference levels for adults [35]. However, by comparing results from the supplemented group with those from the age-matched placebo group we were able to make appropriate adjustments for age-related changes in the statistical analyses.

2.4. Determination of selenium

The Se analyses were performed on serum using the inductively coupled plasma mass spectrometry methodology on an Agilent 7700 platform at Kompetenzzentrum für komplementärmedizinische Diagnostik, Zweigniederlassung der Synlab MVZ Leinfelden GmbH (Leinfelden-Echterdingen, Germany). The clinical serum calibrator used was No. 9928 (lot 538).

The accuracy of the measurements was checked by analysing two external reference materials with certified values of 63 and 103 μg/l (control programme offered by the Society for Advancement of Quality Assurance in Medical Laboratories, INSTAND e.V., Düsseldorf, Germany), showing values within 90 %–110 % of certified concentrations. A round-robin test with INSTAND e.V. was always passed adequately. The precision of the method, checked by repetitive analyses of the same sera, showed an average coefficient of variation of 5.7 %.

2.5. Statistical methods

Descriptive data are presented as percentages or mean ± SD. A student's unpaired two-sided T-test was used for continuous variables and the chi-square test was used for analysis of one discrete variable. As the dataset demonstrated a slight non-Gaussian distribution, the dataset was log-transformed when evaluating continuous variables in order to obtain a normal distribution. The effect of this transformation was controlled through a Kolmogorov-Smirnov test. Transformed data were used in the T-test evaluations as this data evaluation is sensitive to a non-normal distribution of measured values. All evaluations were performed according to the "intention-to-treat" principle.

Repeated measures of variance were used in order to account for the individual changes in the concentration of fructosamine taking place between six months and 42 months, instead of comparing the group mean values of the two time points. Evaluations were performed in the total study population and among subgroups: the non-diabetic group, the diabetic group, those with IHD or hypertension, the two gender groups, and finally those above and below the mean age of the total study population (76 years).

As the analysis of variance (ANOVA) algorithm can handle a slight non-Gaussian distribution, non-transformed data were applied in the repeated measures of variance evaluation. In the analysis of covariance (ANCOVA) evaluation both transformed and non-transformed data were applied, with no significant difference in the results.

In order to validate the results obtained from the repeated measures of variance by also adjusting for important covariates, we conducted an ANCOVA evaluation

In the latter evaluation, the fructosamine concentration after 42 months was used as an independent variable, and adjustments were made for age, smoking, hypertension, IHD, fructosamine concentration after six months, Hs-CRP and for active treatment. *P*-values < 0.05 were considered significant, based on a two-sided evaluation. All data were analysed using standard software (Statistica v. 13.2, Dell Inc, Tulsa, OK).

3. Results

The study population in this sub-study consisted of 219 participants, of which 118 received active treatment, and 101 received a placebo. The baseline characteristics of the population are shown in Table 1. There were no significant differences between the two populations, active treatment and placebo, and thus the two populations were balanced.

3.1. Selenium and coenzyme Q10 levels at the start and at the end of the intervention

At the start of the study the mean serum selenium concentration was 67.1 μg/L (SD 16.8). After 48 months the concentration of selenium in the active treatment group was 210.3 μg/L (SD 59.4). Regarding the concentration of coenzyme Q10 the pre-intervention concentration in the population was 0.82 mg/L (SD0.31), and the concentration in the active treatment group after 48 months was 2.17 mg/L (SD1.33).

3.2. Fructosamine and intervention with selenium and coenzyme Q10

At start of the sub-study, that is after six months, there was no significant difference in fructosamine concentration between the active treatment group, and the placebo group (271 ± 35 μmol/L vs. 268 ± 36 μmol/L; *P* = 0.53). However, after 42 months a highly significant difference between the two groups could be found (265 ± 42μmol/L vs. 280 ± 43μmol/L; *P* = 0.01).

In the group receiving the placebo, a significant increase in the concentration of fructosamine took place (268 ± 36 μmol/L vs. 280 ± 43 μmol/L; *P* = 0.04), whereas the mean values of those

Table 1

Baseline characteristics of the study population receiving dietary supplementation of selenium and coenzyme Q10 combined or placebo during four years.

	Active treatment group N = 118	Placebo group N = 101	P-value
Age years, mean (SD)	76.2 (3.1)	76.3 (3.1)	0.74
Gender			
Males, n (%)	58 (49.2)	43 (42.6)	
Females, n (%)	60 (50.8)	58 (57.4)	
History			
Diabetes, n (%)	20 (16.9)	18 (17.8)	0.87
Smoking, n (%)	8 (0.7)	9 (0.9)	0.56
BMI, mean (SD)	27.4 (3.8)	27.0 (4.2)	0.50
Hypertension, n (%)	81 (68.5)	72 (71.3)	0.67
IHD, n (%)	22 (18.6)	16 (15.8)	0.59
NYHA class I, n (%)	71 (60.2)	58 (57.4)	0.68
NYHA class II, n (%)	29 (24.6)	30 (29.7)	0.39
NYHA class III, n (%)	18 (15.3)	12 (11.9)	0.47
NYHA class IV, n (%)	0	0	
Medications			
Anticoagulants, n (%)	9 (7.6)	9 (8.9)	0.73
ACEI, n (%)	15 (12.7)	14 (13.9)	0.80
ARB, n (%)	4 (3.4)	7 (6.9)	0.23
Beta blockers, n (%)	44 (37.3)	33 (32.7)	0.48
Digitalis, n (%)	5 (4.2)	1 (0.9)	0.14
Diuretics, n (%)	39 (33.1)	33 (32.7)	0.95
Statins, n (%)	27 (22.9)	17 (16.8)	0.27
Examinations			
EF < 40 %, n (%)	7 (5.9)	4 (4.0)	0.51
Atrial fibrillation, n (%)	6 (5.1)	7 (6.9)	0.56
s-selenium pre-intervention µg/L, mean (SD)	66.6 (15.9)	67.4 (17.2)	0.56
NT-proBNP, ng/L, mean (SD)	296 (324)	348 (526)	0.38

Note: ACEI: ACE- inhibitors; ARB: Angiotension receptor blockers; EF: Ejection fraction; IHD: Ischemic heart disease; NT-proBNP: N-terminal fragment of proBNP; NYHA: New York Heart Association functional class; SD: Standard Deviation.

Note : Values are means ± SDs or frequency (percent).

Note: Student's unpaired two-sided T-test was used for continuous variables and the chi-square test was used for analysis of one discrete variable.

receiving active treatment did not change significantly (271 ± 35µmol/L vs. 265 ± 42µmol/L; *P* = 0.22). Thus, in the group receiving the placebo, a significant 4% increase in the concentration of fructosamine took place. In order to validate this "small" difference we applied repeated measures of variance methodology where emphasis is made on the changes found in each participant.

That analysis showed a highly significant difference in fructosamine concentration (*P* = 0.001) (Fig. 1). In order to validate the results and also to adjust for some important covariates, we also conducted an ANCOVA evaluation. There was a significant difference in fructosamine concentration at 42 months between those receiving active treatment and those receiving the placebo (*P* = 0.003), even when adjusting for age, fructosamine concentration after six months, diabetes, smoking hypertension and IHD, and level of Hs-CRP as an indicator of activity of inflammation.

3.3. Fructosamine and the non-diabetic population

In the non-diabetic population, the effect of the intervention on the fructosamine concentration was evaluated. Applying repeated measures of variance, a highly significant difference was found (*P* = 0.002) (Fig. 2). In the ANCOVA model adjusting for age, smoking, hypertension, IHD, Hs-CRP, and fructosamine concentration after six months the effect of the intervention with selenium and coenzyme Q10 remained significant after 42 months (*P* = 0.02).
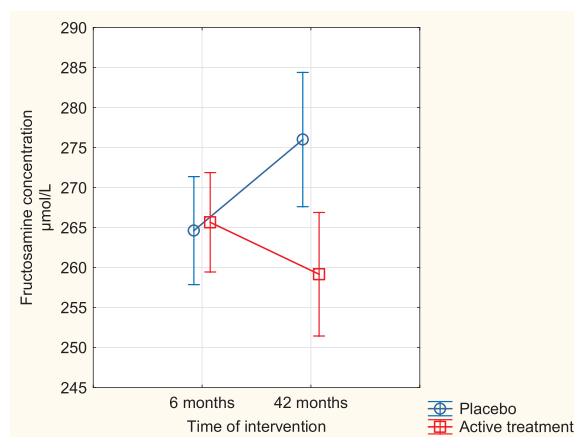
Fig. 2. Concentration of Fructosamine after 6 and 42 months in the placebo and selenium and coenzyme Q10 treatment groups in the non-diabetic population. Note: Evaluation performed by use of repeated measures of variance methodology

Note: Current effect: F(1, 179) = 10.00; p = 0.002

Note: Vertical bars denote 095 confidence intervals

Note: Blue circles: Placebo; Red squares: Active treatment group. Bars indicate ± 95 % CI

3.4. Fructosamine and the diabetic population

The proportion of participants with diabetes was small (n = 38). Applying repeated measures of variance did not reveal any significant differences. However, it was not possible to draw a conclusion about the effect of the intervention in this group, and it is possible that the changes were too small to be detected in the group.

3.5. Fructosamine concentration in those with ischaemic heart disease or hypertension

Evaluating the mean concentration of fructosamine after 42 months of treatment in the group with known IHD or hypertension, a significant difference could be noted, with a higher concentration in the placebo group (280 ± 43 µmol/L vs. 266 ± 45 µmol/L; *P* = 0.04).

Applying repeated measures of variance evaluation to this population, a highly significant difference between those on active treatment compared to those on the placebo could be found (*P* = 0.006). In the ANCOVA model there was a significant difference in fructosamine concentration between those receiving active treatment compared to those on the placebo, also after adjustment of age, fructosamine concentration after six months, diabetes, Hs-CRP, and smoking (*P* = 0.02).

3.6. Fructosamine concentration in males and females

Upon evaluation of the group mean fructosamine concentration difference between those on active treatment and those on the placebo among the males, a borderline significance was observed in fructosamine concentration after 42 months between the active intervention and placebo groups (*P* = 0.06).

However, when applying repeated measures of variance to the same population, a significant difference in fructosamine concentration between those on active treatment and those on the placebo could be demonstrated (*P* = 0.04). The effect of the intervention remained significant even when adjusted for age, fructosamine concentration after six months, smoking, hypertension, Hs-CRP, and IHD in the ANCOVA model (*P* = 0.008).

In the female population, we found no significant difference in the group mean values between those on active treatment and those on the placebo (*P* = 0.08). However, by use of the repeated measures of variance methodology, a significant difference between the two groups

could be found ($P = 0.01$). After adjustment for age, fructosamine concentration after six months, smoking, hypertension, Hs-CRP, and IHD, the significant differences could also be demonstrated in the ANCOVA evaluation ($P = 0.02$).
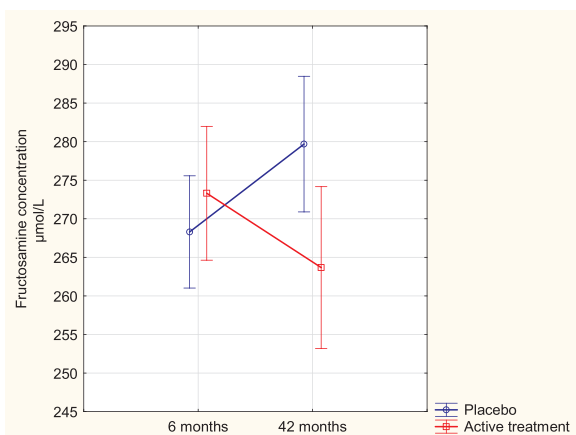
3.7. Fructosamine concentration and age

Upon evaluation of the group mean values in the individuals below the median age (76 years) no significant differences in fructosamine concentration between those on active treatment versus those on the placebo appeared. However, by applying repeated measures of variance a significant difference between the two groups could be found ($P = 0.01$). Applying the ANCOVA methodology, after adjustments for the following covariates: fructosamine concentration after six months, smoking, hypertension, IHD, and Hs-CRP, the active intervention demonstrated a significant difference in fructosamine concentration ($P = 0.02$).

In the group above the median age (76 years), a significant difference was found in concentration of fructosamine between those on active treatment after 42 months, and those on the placebo at the same time ($260 \pm 36\,\mu mol/L$ vs. $275 \pm 43\,\mu mol/L$; $P = 0.04$). Applying repeated measures of variance confirmed the difference (P = 0.03). This difference also persisted in the ANCOVA evaluation after adjustment for smoking, hypertension, Hs-CRP, IHD, and fructosamine concentration after six months, where a significantly lower concentration could be seen in those receiving active treatment ($P = 0.01$).

3.8. Relation between serum selenium concentration at the start of the intervention, and change in fructosamine concentration after the intervention

We also examined whether the impact of the intervention was dependent upon the baseline concentration of selenium in plasma. In the group with an initial selenium concentration below the median concentration we observed a more pronounced decrease in the fructosamine concentration following the intervention with selenium and coenzyme Q10 ($P = 0.0014$) (Fig. 3), as compared to those with a selenium concentration above the median ($P = 0.015$) (Fig. 4) using the repeated measures of variance methodology.
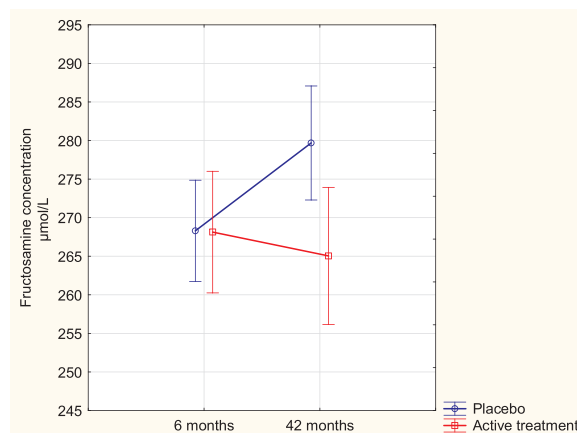

Fig. 3. Concentration of Fructosamine after 6 and 42 months in the placebo, and selenium and coenzyme Q10 treatment group in a population with a pre-intervention serum selenium concentration below median.
Note: Evaluation performed by use of repeated measures of variance methodology
Note: Current effect: F(1,170) = 10.62; p = 0.001
Note: Vertical bars denote 095 confidence intervals
Note: Blue circles: Placebo; Red squares: Active treatment group. Bars indicate ± 95 % CI

Fig. 4. Concentration of Fructosamine after 6 and 42 months in the placebo, and selenium and coenzyme Q10 treatment group in a population with a pre-intervention serum selenium concentration above median.
Note: Evaluation performed by use of repeated measures of variance methodology
Note: Current effect: F(1,169) = 6.10; p = 0.015
Note: Vertical bars denote 095 confidence intervals
Note: Blue circles: Placebo; Red squares: Active treatment group. Bars indicate ± 95 % CI

4. Discussion

This is a sub-study of the main intervention study where selenium and coenzyme Q10 were given for four years as dietary supplements to an elderly community population with a low selenium intake compared to the adequate level of $\geq 100\,\mu g/L$ [23,36]. Since the endogenous production of coenzyme Q10 declines in the old [21], coenzyme Q10 was also supplemented. The main study reported reduced cardiovascular mortality and increased cardiac systolic function after this supplementation. Furthermore, signs of less inflammatory activity and reduced oxidative stress compared with the placebo group could be demonstrated [28,37,38].

In the present study we found that intervention with selenium and coenzyme Q10 was significantly associated with reduced fructosamine concentration compared with the concentration in the placebo group, which tended to increase. However, there was no difference in fructosamine values between placebo and active treatment after six months of intervention, although the impact of the intervention became apparent after 42 months.

In the literature, the fructosamine concentration is positively associated with diabetes and increased blood glucose level [39]. However, an interesting study was presented by Shohat et al. who examined the fructosamine concentration along with glucose and HbA1c in 829 patients undergoing joint arthroplasty [40]. Those with a high fructosamine concentration ($\geq 292\,\mu mol/L$) had a significantly higher frequency of complications after surgery than those with a low fructosamine level, irrespective of whether or not there was simultaneous occurrence of diagnosed diabetes. This could indicate that there are more profound mechanisms activated in those with a high fructosamine concentration compared to those with a low level of fructosamine. Some of these mechanisms could be inflammatory activity or oxidative stress. Thus, Rabbany and Thornally (2016) proposed that oxidative mechanisms are involved in the glycation of proteins [41]. Patients with diabetes present a higher level of oxidative stress, as is documented in the literature [42]. In a large population (n = 215,011) Malmström et al. [43] found an increased mortality also among individuals without diabetes who nevertheless had a high fructosamine concentration. The authors [43] suggested that the explanation for the increased risk for those with fructosamine values falling outside the reference interval in this non-diabetic population could be an

association between fructosamine concentration and the level of inflammation and smoking.

In our study, we adjusted for smoking and one of the most well-known biomarkers for inflammation, Hs-CRP. In spite of the adjustments, we found that the intervention with selenium and coenzyme Q10 was associated with lower levels of fructosamine in the main population as well as in all subpopulations, irrespective of gender, indicating a robust effect of the intervention.

In a recent mouse model of postnatal catch up growth, Tarry-Adkins et al. studied insulin signalling and found that intervention with coenzyme Q10 could prevent the development of insulin resistance [44]. From an intervention study on diabetic patients, Brauner et al. reported reduced inflammation in those who received coenzyme Q10 [45]. Although interventions with coenzyme Q10 show conflicting results, possibly as a result of mono-intervention with coenzyme Q10 [46], it could be hypothesised that a cardio-protective effect of the intervention with selenium and coenzyme Q10 could be expected for those with insulin resistance, metabolic syndrome or type 2 diabetes mellitus. Even though we did not observe significant changes in fructosamine from the intervention with selenium and coenzyme Q10 among the small group of diagnosed diabetic individuals in our study, other reports indicate vascular protection of exogenous antioxidants in diabetic individuals [47].

In the evaluation, the treatment caused a more pronounced decrease of fructosamine concentration in the group with pre-intervention serum selenium below the median value (i.e. 67 μg/L), compared with those having a pre-intervention selenium concentration above the median. A possible explanation is that the supplementation has a more powerful effect on those with selenium deficiency, as compared to those who have a less deficient or adequate selenium status. This concurs with our previous results on cardiovascular mortality (42). In the groups with a more profound selenium deficiency a stronger reduction in cardiovascular mortality was observed following the intervention than in those with less deficiency. In a meta-analysis including more than 13,000 participants, Wang et al. reported a non-linear association between risk of diabetes type II and selenium concentration [48]. This accords with our finding that intervention with selenium caused a stronger reduction in fructosamine among those who had a low pre-intervention selenium concentration.

We hypothesised that when a balanced supply of selenium together with coenzyme Q10 is provided, this would influence both the level of inflammation and oxidative stress. It is proposed that oxidative mechanisms could be involved in the glycation of proteins [49]. Fructosamine is synthesised as a result of the glycation of proteins, hence, supplementation with selenium and coenzyme Q10 resulting in less glycation and possibly less glycoxidation might be one of the mechanisms contributing to a lower cardiac mortality and less inflammation as reported earlier in our study.

The consistent positive effect of the intervention with selenium and coenzyme Q10 on fructosamine concentrations could be another small piece of information that could add to the complex picture of the mechanisms behind the surprising clinical cardiovascular outcomes in this relatively small study. It should be emphasised that both selenium and coenzyme Q10 were supplemented in the study, and it is not possible to identify the effect of each of the two components separately, as a synergistic effect between the two is known from the literature.

The present study has demonstrated an effect of supplementation with selenium and coenzyme Q10 on a population that is low or suboptimal in selenium intake, as in fact the majority of populations are in the European countries. In contrast, supplementation of selenium has not been shown to exert a significant cardio-protective effect in studies on selenium-adequate populations, such as studies conducted on US populations that are selenium replete, e.g. the NPC and SELECT studies [50]

We suggest that our findings should be regarded as hypothesis-generating, and should stimulate further research in the area of protective measures against glycaemic and oxidative stress.

5. Limitations

The studied population was of limited size, 219 individuals, which makes the interpretation of the results difficult. However, as the difference between the two groups, active supplementation versus placebo, was highly significant, it is probable that the results reflect real changes. The report should be regarded as a hypothesis-generating study, and as such it has interesting information that could be used in further research.

The study was performed in Sweden, which, like all European countries, has a low selenium content in the soil and an inadequate dietary supply. The same low selenium levels are also reported from Australia and New Zealand. However, the positive results of the intervention might not be valid in areas and populations where the selenium supply is adequate. Indeed, we observed reduced cardiovascular mortality primarily in the subgroups lowest in selenium at baseline [51].

The study population was not sampled primarily, but were chosen because they lived in the same rural community and in the same age stratum. The results could therefore be subject to bias because of a lower participation threshold among those with known or unknown disease, with impaired well-being, or hoping for a diagnosis or treatment adjustment. This situation could theoretically result in an even higher level of fructosamine in our study population compared with other healthy populations of corresponding age. However, as the main study population was randomised into two groups, we assume that those given active treatment had a similar health situation as those on the placebo, as seen from the balanced presence of covariates in the baseline characteristics.

Also, the study population represented a specific age stratum of middle-aged and elderly, so it is difficult to extrapolate the obtained information into other age groups.

Finally, the study population was an ethnically homogenous Caucasian population; thus, we do not know if the information could be applied to other populations.

6. Conclusion

In this sub-study we observed a significant decrease in fructosamine concentration as a result of intervention with selenium and Q10. Fructosamine, a marker for long-term glycaemic control in diabetic patients. It is also positively associated with risk of cardiovascular diseases both in diabetics and non-diabetics.

The decrease could be seen in both males and females, in both the middle-aged and in the elderly, and also in the non-diabetic group. Those with a lower pre-intervention level of selenium had a more pronounced decrease of fructosamine. The obtained decrease could have contributed to the reduced cardiac mortality, and might reflect less inflammation, as has earlier been reported. We suggest that lower glycoxidation as reflected in lower fructosamine concentration might be one of the important effects that results in a lower level of inflammation and oxidative stress. Achieving these effects is one of the important goals in order to reduce cardiovascular risk in an adult population. This strengthens the clinical need to supplement adult and elderly populations deficient in selenium. However, more research in the area is needed.

Ethical approval

The study was approved by the Regional Ethical Committee (No. D03−176; Forskningsetikkommmitten, Hälsouniversitetet, SE-581 85 Linköping, Sweden) and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. (The Medical Product Agency declined to review 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).

Author contributions

UA, AL, PJ Conceived and designed the research project. UA,and AL conducted the research. AL provided the essential reagents, and performed the analyses. UA and AL analysed data and performed the statistical analyses. UA, PJ, JAa, JA and AL wrote the paper. UA had the final responsibility for the final content.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2020.126541.

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