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Serum selenium predicts levels of F_2-isoprostanes and prostaglandin $F_{2\alpha}$ in a 27 year follow-up study of Swedish men

JOHANNA HELMERSSON[1], JOHAN ÄRNLÖV[1], BENGT VESSBY[1], ANDERS LARSSON[2], GEORG ALFTHAN[3], & SAMAR BASU[1]

[1]*Clinical Nutrition Research/Geriatrics, Department of Public Health and Caring Sciences, Faculty of Medicine, Uppsala University, Uppsala, Sweden,* [2]*Clinical Chemistry, Department of Medical Sciences, Uppsala University Hospital, Uppsala, Sweden, and* [3]*Biomarker Laboratory, Department of Health and Functional Capacity, National Public Health Institute (KTL), Helsinki, Finland*

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Abstract

Low concentrations of selenium (Se) predict mortality and cardiovascular diseases in some populations. The effect of Se on *in vivo* indicators of oxidative stress and inflammation, two important features of atherosclerosis, in human populations is largely unexplored. This study investigated the longitudinal association between serum selenium (s–Se) and a golden standard indicator of oxidative stress *in vivo* (8-iso-prostaglandin $F_{2\alpha}$, a major F_2-isoprostane), an indicator of cyclooxygenase (COX)-mediated inflammation (prostaglandin $F_{2\alpha}$), high sensitive C-reactive protein (hsCRP), interleukin-6 (IL-6) and serum amyloid A protein (SAA) in a follow-up study of 27 years. The s–Se was measured in 615 Swedish men at 50 years of age in a health investigation. The status of oxidative stress and inflammation was evaluated in a re-investigation 27 years later by quantification of urinary 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro- $PGF_{2\alpha}$ (a major metabolite of $PGF_{2\alpha}$) and serum hsCRP, SAA and IL-6. Men in the highest quartile of s–Se at age 50 had decreased levels of 8-iso-$PGF_{2\alpha}$ compared to all lower quartiles ($P < 0.01–0.05$), and decreased levels of $PGF_{2\alpha}$ compared to all lower quartiles ($P < 0.001–0.05$), at follow-up. These associations were independent of BMI, diabetes, hyperlipidemia, hypertension, smoking, α-tocopherol and β-carotene at baseline. The s–Se was not associated with hsCRP, SAA or IL-6 at follow-up. In conclusion, high concentrations of s–Se predict reduced levels of oxidative stress and subclinical COX-mediated (but not cytokine-mediated) inflammation in a male population. The associations between Se, oxidative stress and inflammation, respectively, might be related to the proposed cardiovascular protective property of Se.

Keywords: *Selenium, prostaglandins, isoprostanes, interleukins, human, epidemiology*

Introduction

Low levels of selenium (Se) might be a risk factor for accelerated atherogenesis, development of cardiovascular diseases and mortality in some populations [1–4]. Oxidative stress and inflammation are considered to be contributing components to the progression of atherosclerosis [5,6]. The antiatherogenic effect of Se have been suggested to be mediated via reduced levels of oxidative stress and altered inflammatory status [4], possibly by the action of selenium-containing glutathione peroxidases (GSH-Pxs) [7]. Human clinical studies of the effect of Se status on biomarkers of oxidative stress and inflammation are, however, sparse.

F_2-isoprostanes are free radical catalysed products of arachidonic acid, and have emerged as reliable

Correspondence: S. Basu, Clinical Nutrition Research/Public Health and Caring Sciences, Uppsala University, Kålsängsgränd 10D, PO-Box 609, SE-751 25 Uppsala, Sweden. Fax: 46 18 611 79 76. E-mail: samar.basu@pubcare.uu.se

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indicators of oxidative stress *in vivo* [8,9]. One of the major F_2-isoprostanes, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), is increased in smokers and in several disorders related to development of atherosclerosis [10–14]. The relation between s–Se and 8-iso-$PGF_{2\alpha}$ has not been studied in healthy humans.

Prostaglandins (PGs) and thromboxanes are important mediators of the inflammatory process [15]. They initially derive from arachidonic acid, transform to the short-lived intermediates PGG_2 and PGH_2 by cyclooxygenase (COX) and potentially form a cascade of different biologically potent eicosanoids, such as PGE_2, $PGF_{2\alpha}$, prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) [15,16]. All of these products control various important physiological and pathophysiological processes in the body [17]. $PGF_{2\alpha}$ is a major bioactive prostaglandin formed at sites of inflammation [18] and can be reliably quantified by measurement of 15-keto-dihydro-$PGF_{2\alpha}$, a major metabolite of $PGF_{2\alpha}$ in plasma. 15-Keto-dihydro-$PGF_{2\alpha}$ has been shown to be a potent indicator of COX-mediated inflammatory processes *in vivo* [19–21] and has been related to cardiovascular risk factors [12,13]. The effect of s–Se on the formation of $PGF_{2\alpha}$ has not been studied in human clinical studies.

Further, inflammatory processes can also be studied by quantification of well-established cytokine-mediated products. IL-6, high sensitivity CRP (hsCRP) and SAA have been shown to be predictors of cardiovascular diseases in human studies [22,23] and their relation to Se is therefore of current interest.

The aim of this study was to examine the longitudinal relation between s–Se and an indicator of oxidative stress, 8-iso-$PGF_{2\alpha}$ and inflammatory indicators $PGF_{2\alpha}$, IL-6, hsCRP and SAA in a prospective population-study of Swedish men with 27 years of follow-up.

Materials and methods

Study population

This study is based on study participants from Uppsala Longitudinal Study of Adult Men (ULSAM). This population-based cohort originally started in 1970–73, when all men born in 1920–1924, and living in Uppsala, Sweden, were invited to participate in a health screening at age 50 (2841 men invited, participation rate 82%) as previously described [24]. The cohort was re-investigated after 27 years (in 1997–2001, at age 77) when all men still alive were re-invited ($n = 1398$), and 839 gave their informed consent to participate in the study. Out of the re-investigated men, 615 were eligible for evaluation of inflammation and oxidative stress at age 77 and had s–Se data from the original investigation at age 50 and thus constitute the study population. Additionally, data from a reinvestigation at age 70 have been used in this study (see Figure 1 for outline of the study). The Ethics Committee at Uppsala University approved the study.

Sample collection, biochemical analysis, anthropometric measurements and medical history at age 50 (baseline)

Blood samples were collected and stored in liquid nitrogen before analysis. Body weight and height, blood pressure, fasting blood glucose and serum cholesterol and triglycerides were analyzed in a standardized way as described previously [24]. Se was determined in serum samples, that had been stored in liquid nitrogen for about 15 years, with the use of the graphite-furnace atomic absorption spectrometric method as described by Alfthan and Kumpulainen [25]. In brief, samples were diluted (1 + 9) with a solution containing nickel nitrate and nitric oxide and measured by a standard additions method.

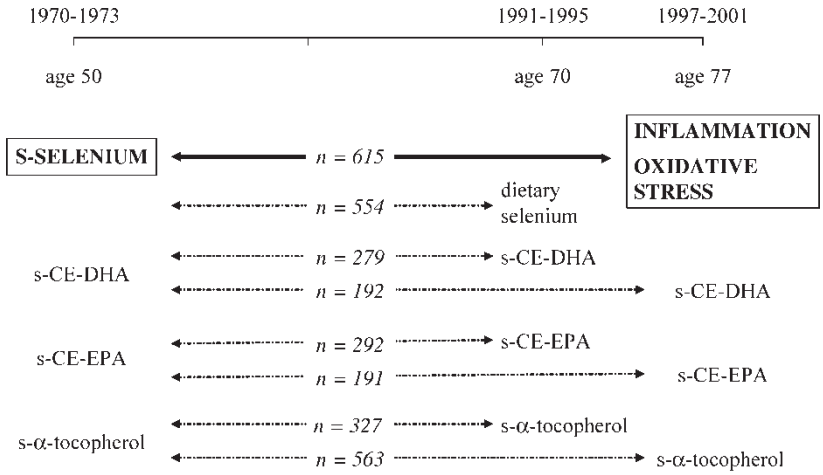

Figure 1. Outline of the study population. The arrows describe the associations studied. s, serum; CE, cholesterol ester, *n*, number of eligible participants for the correlation analysis; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

CV of the method was 4.4% at 87μg/l. Diabetes was diagnosed as fasting blood glucose ≥6.1 mmol/l or anti-diabetic medication. Hypertension was defined as a blood pressure >140/90 mmHg or antihypertensive medication. Hyperlipidemia was defined as a serum cholesterol >6.5 mmol/l or triglycerides >2.3 mmol/l or lipid-lowering medication. Smoking status was evaluated by an interview.

Sample collection, biochemical analysis and medical history at age 77 (27 years of follow-up)

Twenty-four-hour urine was collected. Blood samples were drawn in heparinized tubes in the morning after an overnight fast and plasma was separated. Both urine and plasma were stored at −70°C until analysis. Free 8-iso-$PGF_{2\alpha}$ in urine was analysed by a radioimmunoassay by Basu, as previously described [26]. The intra-assay CV was 14.5% at low and 12.2% at high concentrations. Urinary 15-keto-dihydro-$PGF_{2\alpha}$ was analysed by a radioimmunoassay developed by Basu, as previously described [19]. The intra-assay CV was 12.2% at low and 14.0% at high concentrations. Levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ were corrected for urinary creatinine analyzed as described previously [27]. IL-6 was analyzed by a high sensitivity ELISA kit (IL-6 HS, R & D Systems, Minneapolis, MN). The total CV of the method was 7% and inter-assay CV was 5%. HsCRP and SAA measurements were performed by latex-enhanced reagent (Dade Behring, Deerfield, IL) with the use of a Behring BN ProSpec analyzer (Dade Behring). Intra-assay CV of the hsCRP method was 1.4% at both 1.23 and 5.49 mg/l and of the SAA method 5.9% at 12.8 mg/l and 3.2% at 81 mg/l. One hsCRP (116 mg/l) and one SAA outlier (573 mg/l) were excluded from the statistical analysis. Diabetes was diagnosed as fasting plasma glucose ≥7.0 mmol/l or anti-diabetic medication. Information on clinical history of myocardial infarction and ischemic stroke was obtained from the Swedish Hospital Discharge Registry.

Fatty acids, dietary assessment, vitamin E and β -carotene

The fatty acid composition in serum cholesterol esters (s–CE) was determined with the use of gas–liquid chromatography at age 50 (baseline) and in a random subsample of the study population at the re-investigation at age 70 (20 years of follow-up) and at age 77 (27 years of follow-up). The method has previously been described in detail [28]. The amounts of s–CE eicosapentaenoic acid (EPA) and s–CE docosahexenoic acid (DHA) are presented as the proportion of the sum of the fatty acids analysed. The intake of dietary Se, EPA and DHA were estimated with the use of a 7-day precoded food record at age 70 (20 years of follow-up). The validity and design of the dietary assessment has previously been described in detail [29]. α-Tocopherol and β-carotene at age 50 (baseline) were simultaneously determined by high-performance liquid chromatography in serum samples that had been stored in liquid nitrogen for about 15 years [30]. Serum α-tocopherol at age 70 and 77 were analysed with high performance liquid chromatography, as previously described in detail [12]. Intra-assay CV for the method is 4.5%. α-Tocopherol was corrected for the sum of total cholesterol and triglyceride concentrations. See, Figure 1, for the number of eligible men in each analysis.

Statistical analysis

Variables with skewed distribution, according to Shapiro-Wilks test ($W < 0.95$), were log-transformed to reach normal distribution. Baseline associations between Se and other variables were tested with Pearson's correlation analysis or unpaired *t*-tests. Associations between Se and eicosanoids and cytokines, respectively, were tested in linear regression models with Se as the dependent variable and eicosanoids and cytokines as independent variables. BMI, serum α-tocopherol, β-carotene, CE-EPA and CE-DHA, diabetes, hyperlipidemia, hypertension and smoking at baseline and development of diabetes, myocardial infarction or ischemic stroke during follow-up were considered as potential confounders. Se was entered into the model as a continuous variable and in quartiles. *P*-values <0.05 were regarded as statistically significant. Calculations were performed with Stata 6.0 and 8.2 (Stata Corporation, College Station, TX).

Results

Selenium and baseline characteristics

Linear correlations of s–Se and baseline characteristics are shown in Table I. The s–Se correlated positively with serum β-carotene, α-tocopherol, diastolic blood pressure and cholesterol at baseline. Four percent of the men at age 50 were diagnosed with diabetes mellitus, 21% with hypertension, 63% with hyperlipidemia and 40% were current smokers. The s–Se was not associated with diabetes, hypertension, hyperlipidemia or smoking at baseline ($P = 0.25, 0.33, 0.16, 0.22$, respectively).

8-Iso-$PGF_{2\alpha}$ (indicator of oxidative stress) at 27 years of follow-up

The s–Se at age 50 was inversely associated with urinary 8-iso-$PGF_{2\alpha}$ at follow-up in the linear regression model (see Table II). This association was still significant when adjusted for BMI, diabetes, hyperlipidemia, hypertension, smoking, β-carotene,

Table I. Linear correlations of s–Se and characteristics of study population at baseline (age 50 years), $n = 615$.

	Mean ± SD	*r*	*P*
s–Se (μg/l)	77 ± 14	–	–
BMI (kg/m^2)	25 ± 2.8	0.07	0.07
b-glucose (mmol/l)	4.9 ± 0.6	−0.002	0.96
Systolic blood pressure (mm Hg)	130 ± 16	0.06	0.14
Diastolic blood pressure (mm Hg)	82 ± 10	0.09	<0.05
Triglycerides (mmol/l)	1.8 ± 0.9	−0.03	0.40
s-cholesterol (mmol/l)	6.8 ± 1.3	0.09	<0.05
s-β-carotene (μmol/l)	0.32 ± 0.19	0.12	<0.01
s-α-tocopherol* (μmol/mmol)	3.5 ± 0.6	0.09	<0.05
s–CE–eicosapentaenoic acid, 20:5ω3 (%)	1.4 ± 0.6	0.34	<0.001
s–CE–docosahexaenoic acid, 22:6ω3, (%)	0.71 ± 0.20	0.32	<0.001

s, serum; b, blood, s–CE, serum cholesterol ester.
* Corrected for the sum of cholesterol and triglycerides.

α-tocopherol and s–CE EPA and DHA at baseline and interim diabetes, ischemic stroke and myocardial infarction during follow-up (Table II). Men in the highest quartile of Se at age 50 had decreased levels of 8-iso-PGF$_{2\alpha}$ at follow-up ($P < 0.01$, $P < 0.01$, $P < 0.05$ vs. the three lower quartiles of Se, respectively), see Figure 2. Adjustment with all of the above mentioned potentially confounding variables increased the *P* values to some extent but the associations were still significant.

PGF$_{2\alpha}$ (indicator of COX-mediated inflammation) at 27 years of follow-up

The s–Se at age 50 was inversely associated with urinary 15-keto-dihydro-PGF$_{2\alpha}$ at follow-up in the linear regression model (see Table II). This association did not change when adjusted for BMI, diabetes, hyperlipidemia, hypertension, smoking, β-carotene, α-tocopherol and s–CE EPA and DHA at baseline and interim diabetes, ischemic stroke and myocardial infarction (Table II). Men in the highest quartile of Se at age 50 had decreased levels of 15-keto-dihydro-PGF$_{2\alpha}$ at follow-up ($P < 0.001$, $P < 0.05$, $P < 0.01$ vs. the three lower quartiles of Se, respectively), see Figure 3. These associations did not change after adjustment for all of the above mentioned potentially confounding variables.

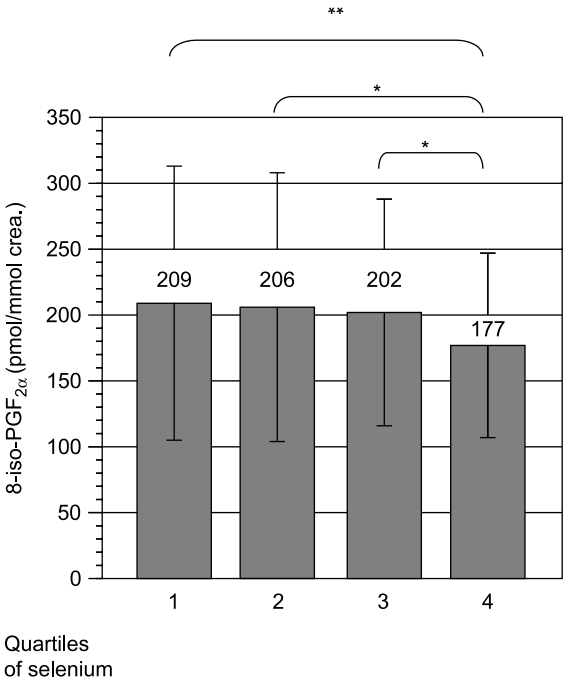

Figure 2. 8-Iso-PGF$_{2\alpha}$ at 27 years of follow-up in quartiles of serum selenium at age 50. *$P < 0.05$, **$P < 0.01$. *P*-values are generated in a linear regression model with Se as a categorical variable. 8-Iso-PGF$_{2\alpha}$ is shown in mean ± SD. Se quartile 1, 30–68 μg/l ($n = 168$); quartile 2, 69–76 μg/l ($n = 144$); quartile 3, 77–85 μg/l ($n = 159$); quartile 4, 86–131 μg/l ($n = 144$).

IL-6, HsCRP and SAA (indicators of cytokine-mediated inflammation) at 27 years of follow-up

The s–Se was not linearly associated with IL-6, hsCRP or SAA at follow-up (see Table II). Quartiles of

Table II. Linear associations between s–Se at age 50 years and indicators of oxidative stress and inflammation at 27 years of follow-up.

	Unadjusted		Adjusted		Adjusted^{antiox}	
	b	P	b	P	b	P
8-iso-PGF$_{2\alpha}$ (pmol/mmol crea.)	−0.048	<0.01	−0.043	0.01	−0.037	0.05
15-keto-dihydro-PGF$_{2\alpha}$ (pmol/mmol crea.)	−0.054	<0.01	−0.053	<0.01	−0.058	<0.01
Hs C-reactive protein (mg/l)	0.001	0.99	–*	–	–*	–
Interleukin-6 (ng/l)	−0.024	0.31	−0.031	0.18	−0.029	0.27
Serum amyloid A protein (mg/l)	0.061	0.09	–*	–	–*	–

Adjusted, adjusted for BMI, diabetes, hyperlipidemia, hypertension and smoking at baseline, and development of diabetes, ischemic stroke and myocardial infarction during the 27 years of follow-up in multiple linear regression models.
Adjusted^{antiox}, additionally adjusted for α-tocopherol, β-carotene, eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3) in serum cholesterol esters at baseline in multiple linear regression models.
b, regression coefficient for 1 SD increase in s–Se; hs, high sensitivity.
* Overall test not significant in the linear regression model. All variables are log-transformed except Se.

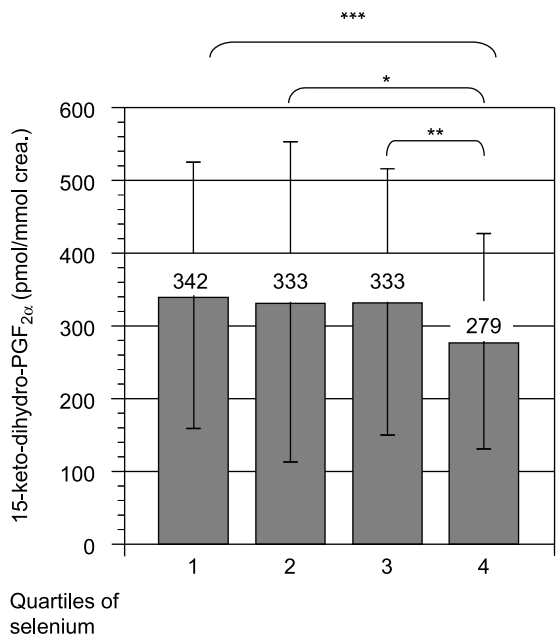

Figure 3. 15-Keto-dihydro-$PGF_{2\alpha}$ at 27 years of follow-up in quartiles of serum selenium at age 50. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. *P*-values are generated in a linear regression model with Se as a categorical variable. 15-Keto-dihydro-$PGF_{2\alpha}$ is shown in mean ± SD. Se quartile 1, 30–68 μg/l ($n = 168$); quartile 2, 69–76 μg/l ($n = 144$); quartile 3, 77–85 μg/l ($n = 159$); quartile 4, 86–131 μg/l ($n = 144$).

Se were not associated to IL-6, hsCRP or SAA in univariate or multivariate regression models (data not shown).

Selenium, dietary assessment, fatty acids and vitamin E

The s–Se at age 50 correlated with reported dietary intake of Se (mean dietary intake of Se 26 ± 8 μg/day) at 20 years of follow-up in the whole cohort ($R = 0.08$, $P = 0.01$, $n = 1001$) but this was not significant if only the study population was included in the correlation analysis ($R = 0.07$, $P = 0.09$, $n = 554$). Se correlated positively with the fatty acid DHA in s–CE at baseline (Table I), and at 20 years of follow-up ($R = 0.14$, $P < 0.05$), but not at 27 years of follow-up ($R = 0.08$, $P = 0.22$). Se correlated positively with s–CE EPA at baseline (Table I), and with s–CE EPA at 20 and 27 years of follow-up ($R = 0.22$, $P < 0.001$; $R = 0.15$, $P < 0.05$, respectively). Further, s–CE DHA at baseline correlated with s–CE–DHA at 20 and 27 years of follow-up ($R = 0.42$, $P < 0.001$; $R = 0.16$, $P < 0.05$, respectively). Similarly, s–CE–EPA at baseline correlated with s–CE EPA at 20 and 27 years of follow-up ($R = 0.42$, $P < 0.001$; $R = 0.25$, $P < 0.001$, respectively). S-α-Tocoperol at baseline correlated well with α-tocopherol at 20 and 27 years of follow-up ($R = 0.32$, $P < 0.001$; $R = 0.35$, $P < 0.001$, respectively).

Discussion

The results from this study showed that men in highest quartile of s–Se at age 50 had significantly lower levels of the oxidative stress indicator 8-iso-$PGF_{2\alpha}$ and the inflammatory indicator $PGF_{2\alpha}$ 27 years later. These results were consistent after adjustment for the most obvious cardiovascular risk factors and other anti-oxidants at baseline.

8-Iso-$PGF_{2\alpha}$, a major F_2-isoprostane, is formed from arachidonic acid in presence of free radicals and is considered as one of the most reliable indicators of oxidative stress *in vivo* [9]. The inverse association between Se and 8-iso-$PGF_{2\alpha}$ found in this study indicate that high Se levels are related to lower levels of oxidative stress. Inverse associations between plasma Se and urinary 8-iso-$PGF_{2\alpha}$ have previously been found in asthmatics and asbestos-exposed subjects [31,32] which support the results in the present study. It is known from animal studies of rats that Se-deficiency accentuate diquat-induced generation of 8-iso-$PGF_{2\alpha}$ [8,33], and rats fed a combination of Se- and vitamin E-deficient diet showed increased levels of F_2-isoprostanes [34].

COX-catalyzed $PGF_{2\alpha}$ is one of the major prostaglandins involved in inflammatory processes. Formation of $PGF_{2\alpha}$ have for several years been difficult to quantify *in vivo*, but the assay for quantification of the $PGF_{2\alpha}$ metabolite (15-keto-dihydro-$PGF_{2\alpha}$) in urine by Basu has made it possible to estimate $PGF_{2\alpha}$ formation in large populations [19]. 15-Keto-dihydro-$PGF_{2\alpha}$ has been shown to be increased in inflammatory conditions *in vivo* and in humans [18–21], including type 2 diabetes and smoking [12,13]. The present study is the first human population-based study evaluating the effect of s–Se on COX-derived $PGF_{2\alpha}$. The results indicate that high concentrations of Se correspond to decreased levels of $PGF_{2\alpha}$. It has been proposed that low Se status is associated with decreased levels of PGI_2 and increased levels of TXA_2, as reviewed earlier [3,4,35]. A Finnish population study showed an inverse relationship between s–Se and platelet aggregability [36], which might be related to altered eicosanoid biosynthesis, although TXA_2 was not evaluated in this study. Rats fed with a Se-deficient diet showed an increased level of $PGF_{2\alpha}$ formation [37], which supports our findings in humans. Further, in experimental acute inflammation, nuclear factor-κB (NF-κB) was increased in Se-deficient macrophages and thereby enhanced COX-2 expression and PGE2-formation [38]. NF-κB might be a central transcription factor in regulating both the free radical and COX-2 pathways.

At low or fairly low intake of Se, as in Sweden and most European countries, plasma Se correlates well with the selenoprotein erythrocyte GSH-Px activity [35]. GSH-Pxs generally act as scavengers of free

radicals or non-radicals, such as hydrogen peroxide and lipid hydroperoxides, hence have antioxidative properties [3,4]. In addition, GSH-Pxs are suggested to be involved in the arachidonic acid metabolism by reducing the hydroperoxide PGG_2 to PGH_2 and influence the formation of bioactive prostaglandins and thromboxanes, hence may regulate inflammation [39]. We speculate that the reduced levels of 8-iso-$PGF_{2\alpha}$ and $PGF_{2\alpha}$ in men with high levels of Se seen in this study might be related to the antioxidative and anti-inflammatory properties of different GSH-Pxs.

The antioxidant and anti-inflammatory properties of the selenoproteins GSH-Pxs *in vitro* have implicated Se-deficiency in the etiology of cardiovascular diseases. The recent discovery of F_2-isoprostanes has facilitated *in vivo* studies of oxidative stress. 8-Iso-$PGF_{2\alpha}$ has been associated with several risk factors for atherosclerosis [10–12,14,40], found in human atherosclerotic lesions [41] and may have a role in the vascular biology of atherogenesis and thereby on inflammatory status. Of COX-derived eicosanoids, mainly TXA_2 and PGI_2 have been implicated in the vascular biology of atherogenesis [42], but since $PGF_{2\alpha}$ has been associated to risk factors for atherosclerosis [12,13], is a potent vasoconstrictor [16] and an important mediator at sites of inflammation [18], the involvement of $PGF_{2\alpha}$ in the vascular biology of atherogenesis can not be excluded. Thus, the results of this present study lead us to speculate that men with high levels of Se and reduced formation of 8-iso-$PGF_{2\alpha}$ and $PGF_{2\alpha}$ might be less susceptible of atherosclerosis.

The relation between Se status and mortality and cardiovascular mortality, respectively, was not reported in this present study. However, previously published data from this Swedish cohort have suggested that low s–Se concentrations predict all cause mortality and also weakly cardiovascular mortality in univariate analysis [43] which is in agreement with studies in other populations where low Se status were independently related to increased risk of cardiovascular diseases [1,2]. Low s–Se was further associated to a greater increase in the carotid intima media thickness in a cohort of Finnish men [44]. However, studies in other populations suggest no relation between Se-levels and cardiovascular diseases [45,46].

The cytokine-mediated inflammatory indicators IL-6, hsCRP and SAA were not related to s–Se in this Swedish population. An inverse correlation was seen between plasma Se and IL-6 in patients with Crohn's disease [47]. The s–Se was inversely associated with C-reactive protein in a healthy American population, indicating a relation between Se and inflammation [48]. Further, Se have been found to be inversely correlated to C-reactive protein during surgery and other pathological conditions [49,50].

The s–Se at baseline correlated with the intake of dietary Se in the re-investigation 20 years later in this present study which could indicate relatively stable dietary habits related to Se-intake in the study participants during follow-up. The s–Se correlated well with the proportion of the fatty acids EPA and DHA in serum cholesterol esters (which in part is likely to be derived from intake of fish) at baseline. This could be expected since it has been estimated that approximately 1/4 of the dietary intake of Se in Sweden is expected to be from fish and seafood [51]. Furthermore, s–Se, s–CE EPA and DHA at baseline correlated well with s–CE EPA and DHA, respectively, at 20 and 27 years of follow-up, and α-tocopherol at baseline correlated well with α-tocopherol at 20 and 27 years of follow-up, which further could indicate that the study participants did not make major changes in their diet during the follow-up period.

This is the first longitudinal, population-based study of the association between Se status and 8-iso-$PGF_{2\alpha}$, the golden standard indicator of oxidative stress and $PGF_{2\alpha}$, a COX-mediated prostaglandin as an inflammatory biomarker. The associations seen could possibly be related to other confounding factors than those we have adjusted for. However, any potential confounding factor introduced during the 27 years of follow-up would bias the results towards the null-hypothesis, i.e. no relation between Se and the biomarkers. The fact that we did find an association between Se and oxidative stress and inflammation, respectively, in spite of the long follow-up suggests that these are clinically relevant findings. Age, sex and ethnicity composition were homogenous in this study, but the results may therefore have limited generalizability to other age and ethnic groups and women. Further, the studied subgroup of the cohort may be healthier than the general population. Although the study has a longitudinal design with a follow-up of 27 years, it can not be excluded that the associations found also would be found in a cross-sectional analysis.

In conclusion, high s–Se concentrations at middle-age predicted reduced levels of oxidative stress (8-iso-$PGF_{2\alpha}$) and reduced levels of COX-mediated ($PGF_{2\alpha}$), but not cytokine-mediated inflammation, in men. These associations between Se, oxidative stress and inflammation, respectively, are important findings which give new fuel into the debate of the proposed anti-atherogenic properties of Se.

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