SPECIAL GUEST EDITOR SECTION

Speciation and Bioavailability of Selenium in Yeast-Based Intervention Agents Used in Cancer Chemoprevention Studies

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This study investigated the speciation and bioavailability of selenium in yeast-based intervention agents from multiple manufacturers from several time points. Sources of selenized yeast included Nutrition 21 (San Diego, CA), which supplied the Nutritional Prevention of Cancer (NPC) Trial from 1981–1996; Cypress Systems (Fresno, CA; 1997–1999); and Pharma Nord (Vejle, Denmark; 1999–2000), which supplied the Prevention of Cancer by Intervention by Selenium (PRECISE) Trial pilot studies. The low-molecular-selenium species were liberated from the samples by proteolytic hydrolysis followed by separation by ion exchange liquid chromatography and detection by inductively coupled plasma-mass spectrometry. The results for the NPC tablets showed that selenomethionine, together with 3 unidentified selenium compounds, were predominant in the sample hydrolysates. The relative amounts of the 4 selenium species varied ($p < 0.05$) among several of the 7 tablet batches used during the course of the NPC Trial. In comparison, 5 batches of more recently produced selenized yeasts, which were used as a source of selenium in the PRECISE and other trials, contained less of the unknown compounds and more selenomethionine at 54–60% of the total selenium in the yeasts. One batch of yeast, however (from 1985), which originated from the same producer as the yeast used in the NPC tablets, contained only 27% of selenium in the sample as selenomethionine. Human subjects receiving 200 μg selenium/day in the UK PRECISE Pilot Trial showed a higher concentration ($p < 0.01$) and higher increase from baseline in plasma selenium than did the same dosage used in the NPC Trial. Differences in intake, speciation, or bioavailability of selenium from the yeast-based supplements in the population groups studied may explain this. Furthermore, the selenium concentration in whole blood from the Danish PRECISE Pilot Trial was higher ($p < 0.001$) than that obtained with synthetic L-selenomethionine in a comparable group of Danes, both groups having been treated with 300 μg selenium/day.

Selenium is an important element in human health. It exerts its essential function as a component of selenocysteine, the 21st amino acid, which is specifically incorporated into a number of selenoproteins with known (e.g., glutathione peroxidases) or unknown (e.g., selenoprotein P) functions (1). When supplemented above the dietary level, which in Northern Europe is on the order of 30–60 μg/day (2), there is evidence that selenium has the potential to protect against some forms of cancer and other human diseases and may prevent the development of AIDS in HIV-positive individuals (3, 4). In an experiment with rats, Ip et al. (5) compared the cancer-protective potential of selenium-enriched garlic and yeast. The most effective cancer preventive effect was observed when the animals were given garlic as the selenium source. This result may exemplify a selenium species-related difference in cancer prevention efficacy, because the major species of selenium in garlic and yeast are γ-glutamyl-methylselenocysteine and selenomethionine (SeMet), respectively. In the Nutritional Prevention of Cancer (NPC) Trial, selenium (as selenized yeast) was used as the intervention agent at 200 μg/day and compared with placebo yeast (6). The study showed a marked decrease in cancer mortality rate and in incident cancers in total and new cases of prostate, colorectal, and lung cancers separately. However, the

Guest edited as a special report on "Inductively Coupled Plasma-Mass Spectrometry: A Powerful Analytical Tool for the Research on Trace Element and Their Species in Food" by Francesco Cubadda.

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individuals in the NPC Trial were high-risk former skin cancer patients. It is difficult to generalize these results directly to healthy individuals. In the planned European Prevention of Cancer by Intervention by Selenium (PRECISE) Trial, which involves healthy individuals, selenized yeast will be the intervention agent. Pilot studies have been conducted in Denmark and the United Kingdom since 1999, where the subjects recruited were randomized to 100, 200, and 300 μg selenium/day or placebo (7). In contrast, the U.S.-based Selenium and Vitamin E Cancer Prevention Trial (SELECT) is using 200 μg/day L-selenomethionine (SeMet) in a factorial design with vitamin E (400 mg/day) and is recruiting a population of healthy men with the primary objective of preventing prostate cancer (4). Although the SELECT Trial is using a pure, chemically synthesized selenium compound, the PRECISE study will use selenized yeast, which contains a range of minor selenium species in addition to the major constituent SeMet, to evaluate the effect of supplementation on cancer incidence. It is therefore important to characterize which selenium species are contained in the selenized yeast intervention agents.

Prior to analysis of selenium species in yeast by liquid chromatography with mass spectrometric (LC/MS) detection, an extraction step is needed. Water was used to extract readily soluble low-molecular-mass selenium species in the cell cytosol (8–11), but the extraction efficiency was low. Proteolytic enzymes have been widely used to hydrolyze amino acid-bound selenium contained in selenium-containing proteins in yeast prior to speciation analysis (12). Alternatively, protein hydrolysis has been performed chemically by hydrochloric acid (13) or methane sulfonic acid, both of which proved more efficient in liberating SeMet from yeast than did proteolytic enzymes (14). When proteolytic hydrolysis was used, typically 55–60% of the total selenium content in the dry yeast was recovered as SeMet. With selenium-selective detection by inductively coupled plasma–mass spectrometry (ICP-MS), more than 30 selenium-containing minor compounds were detected (15). In comparison, when chemical hydrolysis was used, the extraction efficiency of selenium in general increased, and 65% (14) or 73% (13) of the total selenium content in the yeasts tested was extracted as SeMet. The use of proteolytic enzymes for species liberation, however, most closely resembles the physiological conditions in the human intestine, whereas the harsher chemical hydrolysis, although efficient, may cause degradation or alteration of selenium species of physiological importance.

ICP-MS equipped with a dynamic reaction cell (DRC) could detect sensitively, selectively, and without interferences all 6 naturally occurring selenium isotopes, including the most abundant ^{80}Se (16). In combination with high-performance liquid chromatography (HPLC), the hyphenated system was used for selenium speciation in enzymatic digests of yeast with natural (17) or ^{77}Se-enriched isotopic composition (15). The ability of the ICP-DRC-MS instrument to simultaneously determine several selenium isotopes was also useful for the estimation of the absorption rate of selenium from yeast by the enriched stable isotope approach. This required accurate determination of the ^{77}Se enrichment in human urine, plasma, and feces following ingestion of intrinsically labeled ^{77}Se enriched yeast. The absorption rate of selenium from selenized yeast used in the PRECISE Trial (SelenoPrecise, Pharma Nord) was estimated at 89 ± 4% (n = 12) in male volunteers (18).

The possibility that selenium contained in the yeast tablets used in the NPC Trial may have possessed different bioavailability or cancer-preventive efficacy in comparison with selenized yeast used in other similar human studies cannot be excluded. Investigating the species distribution in these tablets is therefore needed and may provide information enabling a critical comparison between the NPC and other selenium-based cancer chemoprevention studies to be made. The purpose of the present study was therefore to characterize the selenium species contained in enzymatic hydrolysates of selenium-yeast tablets used in the NPC Trial and in selenized yeasts used in ongoing human intervention studies. Furthermore, the response to selenium supplementation with different yeasts can be compared.

Experimental

Instrumentation

An ELAN 6100 ICP-DRC-MS instrument (Perkin-Elmer SCIEX, Concord, Ontario, Canada) was used alone for total selenium determinations or hyphenated with cation- or anion-exchange LC for selenium species separation, and detection is described in detail elsewhere (15). The ^{77}Se and ^{80}Se isotopes were monitored by using a radio frequency power of 1350 W, and the MS used a dwell time of 500 ms per isotope in the quadrupole analyzer. The DRC quadrupole was adjusted for removal of the argon dimer interferences by adjusting the settings to the optimum signal-to-noise ratio at m/z 80 of a 10 μg/L standard selenium solution and by using a flow rate of the DRC gas (methane) at 0.6 L/min (16).

For the ion-exchange LC separations, a PE Series 200 metal-free quaternary HPLC pump (Perkin-Elmer, Norwalk, CT) was used in conjunction with a Waters 717 Plus autosampler (Waters, Milford, MA). For the cation-exchange separations, a silica-based strong cation-exchange HPLC column, 100 × 3 mm id (Ionosphere-5C, Chrompack International, Middelburg, The Netherlands), was used in a gradient elution program involving the use of 0.75–8mM aqueous pyridinium formate as mobile phase (15). For the independently conducted anion-exchange separations, a strong polymeric HPLC column, 120 × 4.6 mm id (ION-120, Interaction Chromatography, Mountain View, CA), was used with an aqueous solution of sodium salicylate with tris-(hydroxymethyl)-aminomethane (TRIS) used for pH adjustment as mobile phase (17, 19). The mobile phases were constantly deaerated by bubbling with a low flow of helium. The injected sample volumes were 10–50 μL, and the flow rate of the mobile phase was 1 mL/min. The chromatographic peak areas were evaluated and quantified against external standard curves using TotalChrom software (Perkin-Elmer).

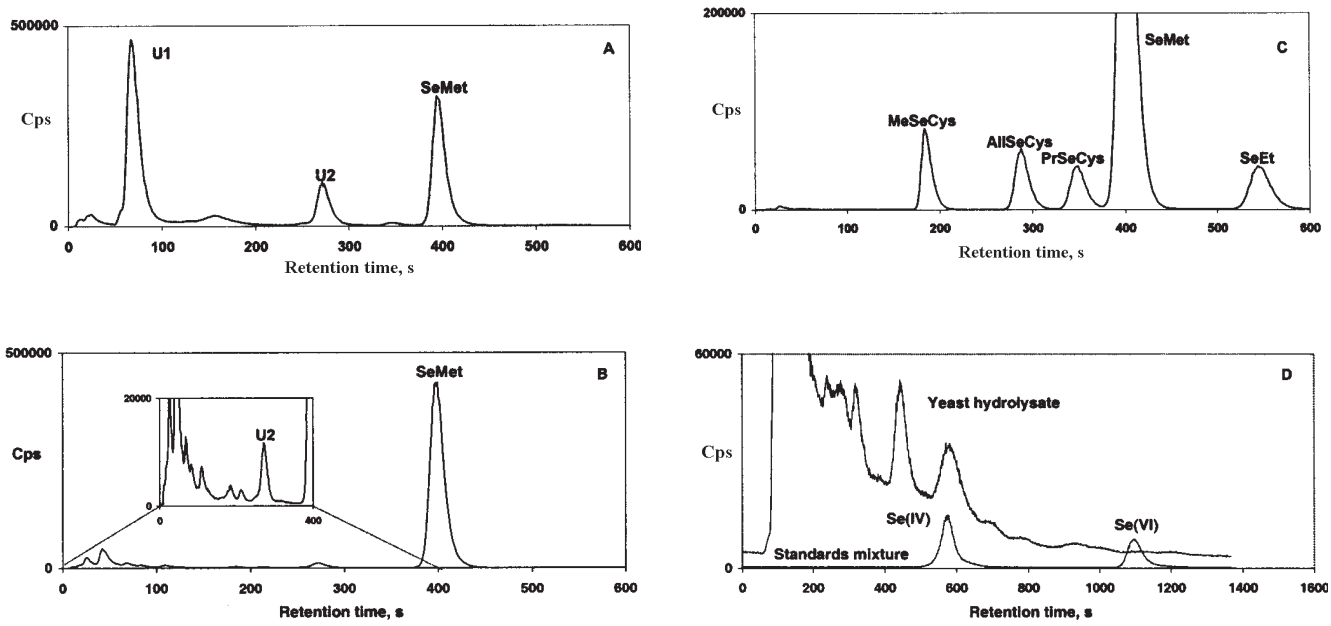

Figure 1. Cation-exchange HPLC-ICP-MS chromatograms of (A) selenized yeast Nutrition 21 (1985), (B) selenized Pharma Nord (batch 23409), (C) standards mixture of selenoamino acids (6.0 μg/mL as selenium of SeMet and 0.6 μg/mL of all other species), and (D) anion-exchange chromatogram of selenized yeast Cypress Systems (batch Se-27) overlaid with chromatogram of a standards mixture of selenite and selenate (40 μg/mL each as selenium). *See* text for peak acronyms used.

Standard Substances and Chemicals

For quantification of the total selenium content, a certified solution of selenium at 1000 μg/mL in 2% nitric acid solution (natural isotopic abundance) was used (CPI International, Santa Rosa, CA). For the selenium speciation work, mixtures of available standards were prepared in aqueous deaerated solution. Se-methyl-selenocysteine (MeSeCys), Se-allyl-selenocysteine (AllSeCys), and Se-propyl-selenocysteine (PrSeCys) were kindly donated by Howard Ganther (University of Wisconsin, Madison, WI). SeMet, selenoethionine (SeEt), sodium selenite (Se (IV)), and sodium selenate (Se (VI)) were obtained from a commercial source (Sigma-Aldrich, Copenhagen, Denmark). Selenomethionine Se-oxide (SeOMet) was prepared in-house by treatment of 5 mL of an aqueous standard of SeMet at 10 μg/mL as selenium by 1–2 mL concentrated (30%, v/v) hydrogen peroxide solution using ultrasound for 10 min. The identity of SeOMet was confirmed by cation-exchange HPLC with electrospray MS detection (17). Water used throughout the work was obtained from a Millipore Element apparatus (Millipore, Milford, MA) and was degassed with He prior to use in order to remove dissolved oxygen. The yeast was pretreated by the proteolytic enzyme Protease XIV (Sigma-Aldrich).

Samples

Subsamples of 7 batches of tablets each containing 200 μg selenium per unit as selenized yeast (Nutrition 21, San Diego, CA) were supplied by author Mary Reid. The samples, which were film-coated, had been stored under dry conditions at room temperature. At the time of analysis the moisture content of the yeasts was 4–6%, m/m. Two batches of selenium-enriched yeasts (Cypress Systems Inc., Fresno, CA) were produced in 1997 and 1999 by inoculation of *Saccharomyces cereviceae* on a substrate of molasses and inorganic selenium. Tablets containing these yeasts as sources of selenium are currently being used in 3 ongoing studies of prostate cancer prevention and cancer progression prevention (20) and other studies in the United States related to lung and breast cancer prevention (personal communication, Henry Thompson, Colorado State University, Boulder, CO). One batch of yeast (Nutrition 21), manufactured in a similar manner in 1985, was included for speciation analysis but had been stored in Denmark since 1986 under nonoptimum conditions, i.e., varying temperature and humidity. Selenized yeasts produced by this manufacturer were used from 1981 to 1996 as the source of selenium in tablets for the NPC Trial (6). Three batches of yeast (SelenoPrecise, Pharma Nord, Vejle, Denmark), produced by inoculation of *S. cereviceae* (strain PN0056) in minimal-substrate containing nutrients of Pharmacopoeia quality and sodium selenite as described in more detail elsewhere (15), were also included. The 3 batches have been or are currently being used in PRECISE pilot studies in the United Kingdom and in Denmark (4, 21).

Sample Pretreatment

All samples were subjected to enzymolysis by Protease XIV (15). Prior to this treatment the tablets were crushed and homogenized manually in a mortar. The yeasts were thoroughly mixed before sampling. A 0.5–0.6 g subsample of the tablets, which corresponded to 200 μg selenium, was taken for

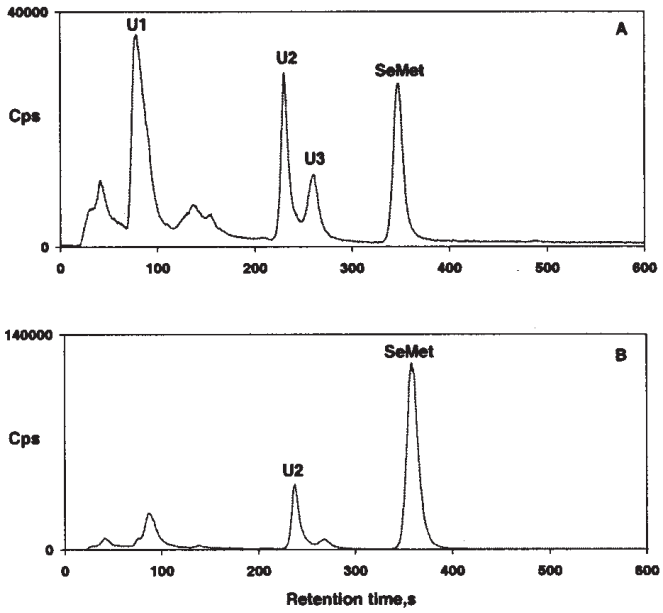

Figure 2. Cation-exchange HPLC-ICP-MS chromatograms of (A) yeast-based tablet Nutrition 21 (batch 10-93) and (B) batch 07-88. For peak acronyms used, *see* text.

analysis, and subsamples of 0.2 g of the yeasts were used. During enzymolysis at 37°C for 24 h, the aqueous sample slurries were stirred constantly. Following this treatment, the samples were centrifuged and the slightly cloudy supernatant was passed through a 0.45 μm pore size disposable filter and diluted 10 times by mobile phase before analysis by HPLC-ICP-MS.

Selenium Determination in Plasma or Whole Blood from PRECISE Pilot Trials

In the UK pilot cohort, lithium–heparin plasma was prepared from whole-blood samples at baseline and after 6 months' supplementation with 0, 100, 200, and 300 μg selenium/day. Plasma samples were stored at –80°C and sent on Dri-ice to Central Science Laboratory (Sand Hutton, York, UK) for selenium analysis by hydride-generation ICP-MS. Plasma samples were prepared for analysis by microwave-assisted digestion by nitric acid (Multiwave, Perkin-Elmer, Bucks, UK), and selenium was reduced to the Se (IV) oxidation state by treatment with hydrochloric acid for 1 h at 90°C before being diluted to volume for analysis. All reagents were of "Analar" grade (or better), and the water used was of Millipore-grade (18 MΩ/cm). The quality control procedures during the preparation and analysis of the samples were accredited under the UK Accreditation Scheme. Accuracy was ensured by analysis of certified reference materials, and the results found for Seronorm Serum were (mean and SD) 85.5 ± 10.9 ng/g (n = 10, certified 86 ng/g); and for NIST 1598 Bovine Serum 43.5 ± 2.7 ng/g (n = 16, certified 42.4 ± 3.5 ng/g). The limit of detection (LOD) was 5 ng/g.

In the Danish pilot cohort, the whole-blood sample was drawn into a lithium–heparin tube, stored at –80°C until shipment to Laboratorium für Spektralanalytische und Biologishe Untersuchungen (Stuttgart, Germany) for analysis of total selenium. A 500 μL subsample was solubilized by 2 mL of a mixture of nitric acid and hydrogen peroxide at room temperature. After

Table 1. Retention times (absolute and relative to selenomethionine) of selenium species in cation-exchange HPLC of extracts of selenized yeast and selenized yeast tablets[a]

	U1		U2		U3		SeMet
	Retention time		Retention time		Retention time		Retention time
	Seconds	Relative	Seconds	Relative	Seconds	Relative	Seconds
Sample type	Mean ± c.i.	Mean ± c.i.	Mean ± c.i.	Mean ± c.i.	Mean ± c.i.	Mean ± c.i.	Mean ± c.i.
Yeasts	65 ± 1.0	0.170 ± 0.002	262 ± 3.4	0.683 ± 0.008	Not detected		384 ± 3.3
Tablets	85 ± 11	0.238 ± 0.018	237 ± 17	0.666 ± 0.005	269 ± 35	0.756 ± 0.079	356 ± 24

[a] Values are given as mean and 95% confidence intervals (c.i.).

Table 2. Quantitative analytical results for selenium and selenium species in yeast and yeast fractions[a]

		Selenium concentration (μg/g) in yeast sample						
			Total chromatographed Se		Selenomethionine		Se(IV)	
Selenized yeasts	Batch ID	Total Se[b]	Mean ± c.i.	Rel. amount, %[c]	Mean ± c.i.	Rel. amount, %[c]	Mean ± c.i.	Rel. amount, %[c]
Cypress Systems	Se 21	1250	1003 ± 124	80	754 ± 41	60	9 ± 7	1.0
Cypress Systems	Se 27	1290	1014 ± 125	79	747 ± 40	58	9 ± 7	1.0
Nutrition 21	—	1750	1553 ± 191	89	466 ± 25	27	7 ± 5	0.4
Pharma Nord	14649	1250	840 ± 103	67	681 ± 37	54	3 ± 2	0.3
Pharma Nord	14650	1300	887 ± 109	68	712 ± 38	55	3 ± 2	0.2
Pharma Nord	23409	1430	1062 ± 131	74	856 ± 46	60	2 ± 2	0.2

[a] Species concentrations are given as mean and 95% confidence interval (c.i.) estimated from double determinations of all samples.
[b] Manufacturer's information.
[c] Relative to total selenium concentration in yeast.

several hours, an additional 100 μL hydrogen peroxide was added to the mixture. The selenium concentration was determined by Zeeman-corrected graphite furnace atomic absorption spectrometry using the 196.0 nm resonance wavelength. Copper and magnesium nitrate were used as matrix modifiers. The accuracy of the selenium analyses was controlled by Seronorm Trace Elements Whole Blood Level 1 (certified 82 ng/g) and Level 2 (certified 126 ng/g), and the results found were (mean and SD) 82.3 ± 6.2 ng/g (n = 10) and 127 ± 8 ng/g (n = 10), respectively. The LOD of selenium was 6.2 ng/g.

Results and Discussion

Qualitative Selenium Speciation by Cation- and Anion-Exchange HPLC-ICP-MS

Cation- and anion-exchange HPLC were useful for the separation of a variety of organic and inorganic selenium species occurring in enzymatic hydrolysates of yeast. The independent use of the cation- or anion-exchange chromatographic systems, although more labor-intensive, ensured improved chromatographic selectivity. This was useful because more than 30 selenium species, including SeOMet, had been separated and detected in yeast (15, 17). The chromatographic selectivity of the cation-exchange HPLC system for selenoamino acid separation is shown for a mixture of standards in Figure 1C. The retention time of SeOMet, which was about 1600 s, was off-scale in the chromatograms shown. Chromatographic peaks corresponding to SeMet and to species of unknown identity (U1 and U2) occurred in the chromatograms of the yeast extracts (Figure 1, A and B). Peaks with the same retention times, in addition to another unknown (U3) peak, occurred in the tablet chromatograms, as shown in Figure 2. SeEt, which was used in the standards mixture, was not detected in any of the samples. The separation of selenite and selenate in the presence of a large quantity of organic selenium species in the yeast hydrolysates, was successfully achieved by anion-exchange HPLC, as shown in Figure 1D.

The qualitative analysis of the selenium species relies on retention time matching with available standard substances. Unfortunately, poor reproducibility of the retention time of peaks in cation-exchange chromatography was observed, in particular during analysis of tablet extracts. This was possibly caused by dissolved tablet matrix constituents that compete for the active sites on the stationary phase of the cation-exchange HPLC system. Table 1 shows that when the retention time was normalized to that of SeMet, the uncertainty was reduced, and the mean retention time for U2 became identical for all yeasts and tablets. For U1, however, the mean normalized retention times were significantly different, which casts doubt on whether U1 in yeasts and in tablets are indeed the same compound. The retention times for SeMet were, within the uncertainty, the same for both sample types. It is concluded that U2 and SeMet were present in all samples.

Quantitative Results and Relative Distribution of Unknown Selenium Species in Yeasts and Tablets

The quantitative results, which were estimated by using calibration curves constructed from the corresponding standard substances, are given for the yeast samples in Table 2. The chromatographed amount of selenium (estimated from total area of chromatogram) using cation-exchange HPLC separations corresponded to 67–89% of the total content in the samples. The maximum value was found for the Nutrition 21 yeast sample. The concentration of selenium as SeMet corresponded to 54–60% of the total selenium content in 5 out of 6 samples, but the SeMet value was low for the Nutrition 21 sample at only 27% of the total selenium content. The major amount of selenium in this sample is therefore of unknown identity. The corresponding chromatogram in Figure 1A shows that the species pattern is different from that found in a more recently produced batch of selenized yeast (Figure 1B).

Table 3. Selenium species in enzymatic hydrolysates of selenized yeast and selenium yeast tablets[a]

Sample			U1	U2	U3	SeMet	SeOMet	Residual
Producer	Year	Batch ID	Percent mean ± c.i.	Percent mean ± c.i.	Percent mean ± c.i.	Percent mean ± c.i.	Percent mean ± c.i.	Percent mean
Selenized yeast tablets								
Nutrition 21	1983	11–83	24.2 ± 0.7	7.8 ± 2.7	3.2 ± 1.1	59.6 ± 4.9	ND[b]	5.2
Nutrition 21	1988	07–88	14.7 ± 0.4	15.4 ± 5.4	3.5 ± 1.2	61.2 ± 5.0	ND	5.2
Nutrition 21	1989	09–89	20.2 ± 0.6	11.4 ± 4.0	3.8 ± 1.3	57.6 ± 4.7	ND	7.1
Nutrition 21	1990	07–90	7.5 ± 0.2	9.0 ± 3.1	5.9 ± 2.1	69.1 ± 5.6	ND	8.6
Nutrition 21	1991	10–91	8.7 ± 0.3	19.8 ± 6.9	1.9 ± 0.7	65.9 ± 5.4	ND	3.7
Nutrition 21	1992	10–92	17.0 ± 0.5	23.9 ± 8.4	2.7 ± 0.9	47.8 ± 3.9	ND	8.6
Nutrution 21	1993	10–93	35.2 ± 1.1	14.6 ± 5.1	9.0 ± 3.1	18.0 ± 1.5	ND	23.1
Selenized yeasts								
Cypress Systems	1997	Se21	3.7	5.1 ± 1.8	< 0.1[c]	75.3 ± 6.2	0.8 ± 0.7	15.2
Cypress Systems	1999	Se27	4.2	5.2 ± 1.8	< 0.1	73.7 ± 6.0	0.2 ± 0.2	16.8
Nutrition 21	1985	—	41.0 ± 1.4	10.8 ± 3.7	< 0.06	30.0 ± 2.5	2.7 ± 2.3	15.5
Pharma Nord	1999	14649	2.5 ± 0.1	2.6 ± 0.9	< 0.1	81.0 ± 6.6	0.6 ± 0.5	13.4
Pharma Nord	1999	14650	ND	2.2 ± 0.8	< 0.1	80.3 ± 6.6	0.2 ± 0.2	17.2
Pharma Nord	2000	23409	0.5	2.0 ± 0.7	< 0.1	80.7 ± 6.6	0.5 ± 0.4	16.4

[a] Results are given as percent peak area relative to total area of chromatograms. Mean values and 95% confidence intervals (c.i.) were estimated from double determinations of all yeasts. U1, U2, and U3 designate peaks of unknown identity. For acronyms used, *see* text. Residual indicates the sum of peak areas from all other selenium species.

[b] ND = Not determined.

[c] < = Less than LOD estimated from 3 times the baseline noise.

The fact that the producer of the former sample provided selenium yeast used as tablets for the NPC Trial (6) triggered our interest in investigating the selenium species contained in the selenium yeast tablets used in that study. The data in Table 2 additionally show that the concentration of selenite did not exceed 1% of the total selenium in the yeasts. This reflected that selenite was efficiently metabolized to organic species by the yeast cells during fermentation. Chromatographic peaks with a retention time of <600 s (Figure 1), which correspond to the region where selenoamino acids eluted, accounted for more than 84% of the total chromatographic area of the cation-exchange chromatograms. This region was studied in this work, whereas the minor proportion of more cationic selenium species, which was eluted at later retention times (15), did not show much between-sample variation and was therefore not studied any further.

Results for the relative amount (peak area as percent of total area of chromatogram) of species of unknown identity (U1–U3) and of SeMet in the yeasts and tablets are shown in Table 3. For the yeast-based tablets the results showed significantly different ($p < 0.05$) abundances of U1, U2, and U3 between several of the batches. This has been illustrated by chromatograms corresponding to the uncharacteristic tablet batch ID 10–93 and to tablet batch ID 07–88 in Figure 2 (A and B). The extraction efficiency for the yeast-based tablets, expressed as the total chromatographed quantum of selenium divided by the total selenium content in the tablets, was on average 60% and ranged between 47 to 74%. This efficiency was somewhat poorer than that reported for the yeasts in Table 2. For the yeast samples, SeMet was the predominant species in 5 out of 6 samples, but in the Nutrition 21 yeast (1985), U1 was predominant in the hydrolysate. In some yeast extracts,

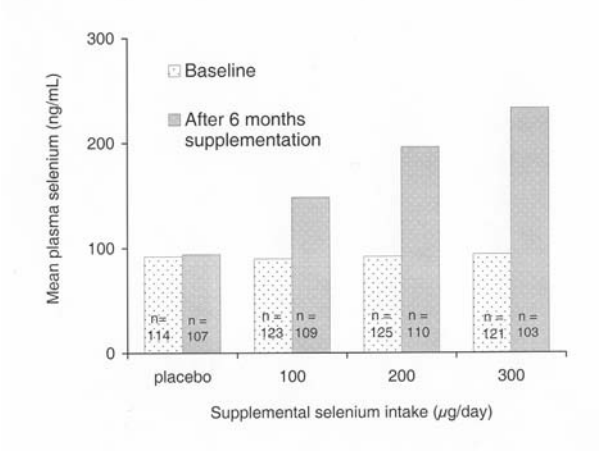

Figure 3. UK PRECISE Pilot Trial: Mean plasma selenium concentration at time zero (baseline concentration) and after 6 months of supplementation, sorted by treatment group receiving placebo, or selenium at 100, 200, or 300 μg/day as SelenoPrecise yeast. The number of samples is indicated on each column.

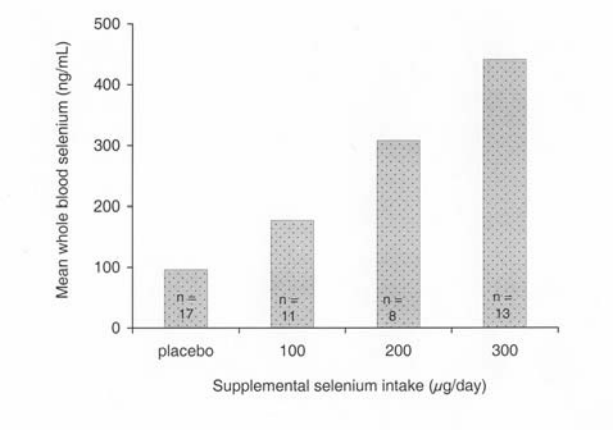

Figure 4. Danish PRECISE Pilot Trial: Mean whole-blood selenium of treatment group after 2 years supplementation by placebo or selenium at 100, 200, or 300 μg/day as SelenoPrecise yeast. The number of samples is indicated on each column.

U1 was incompletely separated from other selenium-containing compounds (*see* inset in Figure 1B), which hampered its analysis. The unknown U3, which occurred in the tablet extracts, was below the LOD in all yeast extracts. The finding of SeOMet, which is likely to be an analytical artifact (15), was detectable in the yeasts at low abundance but was not searched for in the tablets.

Bearing in mind that the PRECISE Trial was based on the results of the NPC study, it is noteworthy that the occurrences of selenium species in the Pharma Nord and in the Cypress Systems yeast batches were similar (Table 3) but differed markedly from that of several tablet batches used in the NPC Trial. Whether this may have an impact on any cancer preventive efficacy of the 2 sets of intervention agents is not known. Identification of the unknown species in the tablet batches is important for understanding the mechanisms by which the selenized yeast supplements may have reduced cancer and mortality rates in humans (6).

Plasma and Blood Selenium Values in the UK and Danish PRECISE Pilot Trials Following Supplementation by Selenium Yeast

Information on selenium species contained in the yeast preparations used in intervention trials is important for understanding and interpretating the observed effects. Furthermore, such information is important when establishing future public health recommendations in order to achieve optimum disease-preventive effect. The results in Table 3 show different patterns of selenium species contained in the intervention agents used in the NPC and PRECISE Trials, suggesting that the response to supplementation, as seen in plasma and blood, may not be comparable.

In the UK Pilot cohort of the PRECISE Trial, selenium levels were determined in blood plasma of 483 participants aged 60–74 years, at baseline (0 months) and after supplementation for 6 months from the time of randomization. The plasma selenium concentrations in Figure 3 corresponded to a dietary selenium intake of approximately 40 μg/day plus 0, 100, 200, or 300 μg/day of selenium supplementation (SelenoPrecise yeast, Pharma Nord, batch 23409). Plasma selenium increased from a baseline value of (mean ± SD) 92 ± 20 ng/mL to 148 ± 28, 196 ± 42, and 233 ± 54 ng/mL in the 100, 200, or 300 μg/day treatment groups, respectively. The plasma selenium levels rose in a dose-dependent, nonlinear fashion, and the resulting plasma selenium concentrations were significantly different among all groups ($p < 0.0001$).

In the Danish PRECISE Pilot cohort, which involved 496 participants in the same age bracket, whole blood levels were determined in blood sampled from 49 randomly selected individuals at the 2-year followup visit. The results in Figure 4 correspond to a dietary selenium intake, which in Denmark has been estimated at a mean value of 48 μg/day (2), plus 0, 100, 200, or 300 μg/day of selenium supplementation. During the first 6 months, Cypress Systems yeast (batch Se-21) was used, and during the last 18 months SelenoPrecise yeast (batch 23409) was used. The selenium levels in whole blood were (mean ± SD) 96 ± 9, 177 ± 18, 308 ± 78, and 441 ± 132 ng/mL for placebo and the 100, 200, and 300 μg/day treatment groups, respectively. The selenium concentration in blood was linearly proportional to the total selenium intake (food intake plus supplement intake) using a standard formula:

$$\text{Blood-Se concentration (ng/mL)} = [(1.16\ (\text{total} \times \text{daily Se intake})\ \mu\text{g/day})] + 29;\ r = 0.884\ (n = 49)$$

The plasma and whole blood selenium values from the 2 PRECISE Pilot Trials were obtained from European population groups with a markedly lower dietary selenium intake than that of NPC Trial subjects whose intake was approximately 90 μg/day (21). In that study the plasma selenium levels increased from a baseline value of (mean ± SD) 114 ± 23 ng/mL to 180 ± 41 ng/mL after 6 months of selenium supplementation by 200 μg/day. The increase in plasma selenium level of 66 ng/mL corresponded to 58% of the baseline level. After supplementation for about 1 year, plasma selenium reached steady state, achieving mean values in the range of 180 to 200 ng/mL. Seven batches of yeast-based tablets, which contained a variable pattern of 4 predominant selenium species (Table 3), were used over the 15 years of the study. It has not been possible to specify which batches corresponded to which plasma levels, although this analysis, as well as a more detailed study of the plasma selenium response to oral intake of selenium, is planned for the near future. In comparison, the mean plasma selenium level of 196 ± 42 ng/mL, which was reached after 6 months supplementation by 200 μg/day in the UK PRECISE Pilot Trial, was, in spite of the lower dietary selenium intake, significantly higher ($p < 0.001$) than that obtained in the NPC Trial and suggests different uptake rates of selenium from the preparations used. The increase in the concentration of selenium in plasma from the UK subjects was 105 ng/mL, or 114% of the baseline level. This was larger than that found in the NPC Trial but was ob-

tained in subjects with a lower plasma selenium level at baseline.

The increase in whole-blood selenium concentrations reached in the Danish PRECISE Pilot Trial after 2 years, supplementation by 300 μg/day was 345 ng/mL or 359% over placebo (Figure 4). In comparison, a study by Clausen et al. (22) used supplementation with selenium at 300 μg/day as synthetic L-SeMet for 1 year in a group of 97 Danish men and women aged 75 ± 8 years. The resulting whole-blood selenium concentration was 375 ± 185 ng/mL, and the increase was 265 ng/mL or 241% over placebo. The mean whole-blood selenium concentration obtained with synthetic L-SeMet was lower ($p < 0.001$) than that obtained with the same daily level of supplementation by selenium yeast in the PRECISE Trial and suggests different degrees of utilization of the yeast-based and the synthetic supplements.

Yeast is a source of multiple organic selenium species, and the predominant one is SeMet. Several of the other unknown species may have clinical importance in the prevention of disease and the promotion of health. It still remains to be seen whether a single substance or a mixture of several selenium species produces the most efficacious results. More information from long-term clinical trials in healthy subjects is required to determine the role of selenium and selenium species in disease prevention. Finally, the production of selenized yeast of highly reproducible composition that could be used throughout the entire time course of an intervention trial is a desirable aim.

Acknowledgments

We thank Kim Overvad and Søren Cold for permission to use data for selenium in blood from the Danish PRECISE Pilot cohort.

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