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Dietary supplementation with selenomethylselenocysteine produces a differential proteomic response☆

Andrea V. Mahn[a,*], Hector M. Toledo[b], Manuel Ruz[c]

[a]Department of Chemical Engineering, University of Santiago of Chile, Santiago, Chile
[b]Program of Cellular and Molecular Biology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile
[c]Faculty of Medicine, Department of Nutrition, University of Chile, Santiago, Chile

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Abstract

Organic forms of selenium offer important health benefits including cancer prevention. Selenium intake has been traditionally quantified as glutathioneperoxidase activity or selenium concentration in blood or tissues. However, these indexes do not reflect organic selenium intake. Effect of dietary supplementation of rats with selenomethylselenocysteine on the blood plasma proteome was investigated in order to detect protein abundance differences between experimental (supranutritional selenium supplementation) and control [minimum selenium dose and sodium selenate instead of selenomethylselenocysteine (SeMSeCys)] groups. Four experimental groups and six control groups consisting of six rats each were fed with base diet supplemented with SeMSeCys or sodium selenate in different concentrations for different periods of time. A proteomic approach, comprising two-dimensional gel electrophoresis and mass spectrometry, was used to assess protein abundance in blood plasma. Statistically significant differences in the abundance of some proteins were detected in all the experimental groups. Four proteins were found to increase their abundance in response to the experimental conditions: apolipoprotein E, haptoglobin and α-1-antitrypsin abundance was related to the extent of supplementation period and transthyretin in response to SeMSeCys dose. Apolipoprotein E and transthyretin were not differentially expressed when diets were supplemented with sodium selenate instead of SeMSeCys. We postulate that these proteins are potential biomarkers of chemoprotective selenium intake.

Keywords: Chemoprotective selenium form; Plasma proteome; Nutriproteomics

1. Introduction

1.1. Selenium and cancer chemoprotection

Selenium is an essential micronutrient for animals and humans, offering important health benefits including cancer prevention. It has been reported that selenium protects against certain cancer types, such as colon cancer [1–3], breast cancer [4], skin cancer [5], prostate cancer [6] and lung cancer [7,8]. Selenium chemoprotection has been attributed to the antioxidant effect of some selenoenzymes [9]. Interventions with selenium have resulted in reduced cancer incidence and mortality. Such effect seems to be stronger in individuals with deficient selenium status [10].

Current investigations on the relationship between selenium intake and cancer prevention have focused mainly upon the chemical form of selenium: selenium-enriched food and in vitro effect of monomethylated forms of selenium. Most of these studies have added sodium selenite or selenomethionine in the diet and concluded that selenite shows a greater chemoprotective effect than selenomethionine [11]. Other studies have established that dietary supplementation with selenized vegetables, such as garlic and broccoli, resulted in a much higher chemoprotective effect, in comparison with selenite and selenomethionine [1,3,12]. These vegetables store inorganic forms of selenium and convert them to an organic form, mainly selenomethylselenocysteine (SeMSeCys), the main compound thought to be responsible for chemoprotection [13].

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* Corresponding author. Tel.: +56 2 7181833; fax: +56 2 6817135.
E-mail addresses: amahn_2000@yahoo.es, amahn@usach.cl (A.V. Mahn).

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1.2. Nutritional biomarkers and selenium intake

Biomarkers reflect metabolic status of a nutrient and provide useful information about metabolism and nutritional status of that nutrient in an organism [14,15]. Selenium bioavailability has been usually determined as selenium saturation concentration in tissues, total selenium concentration in blood and also as glutathione peroxidase activity. Despite that these methods are widely accepted, recent findings strongly suggest that alternative methods to detect organic forms of selenium intake or bioavailability are necessary. Selenium chemoprotective capacity seems to have no relation with glutathione peroxidase activity or with residual selenium concentration in tissues [12]. Besides, no correlation exists between colon cancer prevention in rats and residual selenium concentration or glutathione peroxidase activity [1].

Alternative measurements of selenium bioavailability that are based on the assessment of the activity of other selenoproteins have been proposed [16,17]. However, their use as selenium biomarkers has not been demonstrated quite likely because of analytical requirements and low reproducibility. A genomic approach has been used to investigate the in vitro effect of different forms of selenium on gene expression in cancer cell lines [18]. Genes involved in apoptosis and molecular mechanisms associated to cancer prevention were found to be induced by selenium. Complementary approaches, such as proteomics and metabolomics, are needed in order to validate these results.

In the present study we used a proteomic approach to identify a protein expression pattern in blood plasma that reflected dietary supplementation with SeMSeCys, a chemoprotective form of selenium. A characteristic protein profile associated with the intake of chemoprotective doses of organic forms of selenium could be useful as a diagnostic tool to asses the nutritional status of individuals regarding their susceptibility to develop certain cancer types. The aim of this work was to identify one or more proteins that could be considered as indicative of bioactive selenium intake. This is the first attempt to directly identify proteins representing a response to SeMSeCys dietary supplementation, by using a proteomic approach.

2. Methods and materials

2.1. Experimental design

Six experimental groups, each one consisting of three female and three male 21-day-old Wistar rats, were fed with Torula Yeast based diet (Dyets Inc, Bethlehem, PA, USA) supplemented with either 0.15 μg selenium (as sodium selenate)/g diet (groups A and D), 1.0 μg Se (as SeMSeCys)/g diet (groups B and E) or 1.9 μg Se (as SeMSeCys)/g diet (groups C and F), during 3 (groups A, B and C) and 10 weeks (groups D, E and F). Groups A and D were the control groups for each period of time. Four additional control groups were included as selenium type controls, using the same conditions as groups B, C, E and F but being exposed to sodium selenate instead of SeMSeCys. This inorganic form of selenium shows no clear correlation with chemoprotective effect [1,3,12]. Table 1 summarizes the various experimental treatments. The doses of selenium were selected on the basis of the chemoprotective effect taking place when supranutritional doses are fed [19]. A dose of 1.0 μg Se/g diet is the maximum recommended dose and 2.0 μg Se/g diet is the lower toxicity limit [20]. In order to prevent toxic effects on the experimental animals, a dose of 1.9 μg Se/g diet was the highest dose used in the study. On the other hand, doses of SeMSeCys close to 2.0 μg Se/g diet have been shown to inhibit by 50% the chemically induced mammary cancer in rat models [21]. Animals were maintained in stainless steel cages. They had free access to deionised water and received the corresponding diet ad libitum. Temperature was maintained at 20°C. Day/night cycles of 12 h were kept.

2.2. Blood plasma samples

After the experimental periods (3 or 10 weeks), blood from each rat was collected by cardiac puncture, using standard protocols [22]. For each rat, 0.5 ml blood was collected in EDTA tubes (BD Biosciences, San José, CA, USA) and 0.5 ml blood in heparine tubes (BD Biosciences), inverted up and down 10 times and immersed in an ice bath. The tubes were then centrifuged at 1300 RCF at 4°C for 10 min. Plasma was separated from the solids. Supernatant was transferred to a new centrifuge tube and was centrifuged at 2400 relative centrifugal force (RCF) at 4°C for 15 minutes in order to discard microplatelets. Plasma samples were kept at −80°C until analysis. Plasma samples of the animals from each experimental group were pooled together in order to normalize eventual interindividual variations among the animals. Albumin was depleted with the Qproteome Murine Albumin Depletion Kit (QIAGEN, Hilden, Germany),

Table 1
Experimental design

Group	Selenium dose (μg Se/g diet)	Selenium form	Extent of the supplementation period (weeks)
A	0.15	Sodium selenate	3
B	1.0	SeMSeCys	3
C	1.9	SeMSeCys	3
D	0.15	Sodium selenate	10
E	1.0	SeMSeCys	10
F	1.9	SeMSeCys	10
G	1.0	Sodium selenate	3
H	1.9	Sodium selenate	3
I	1.0	Sodium selenate	10
J	1.9	Sodium selenate	10

Groups A and D are the time controls, Groups I and J are the selenium-type controls and groups B, C, E and F are the experimental groups. Two forms of selenium were used: SeMSeCys as an organic form, and sodium selenate as an inorganic form. The control diet consisted in a base diet with the minimum recommended selenium dose. Two periods of supplementation time were studied: 3 and 10 weeks.

following the manufacturers instructions. Albumin depletion is a highly reproducible procedure that is frequently used in proteomics approaches to biomarkers discovery [23]. Protein concentration was determined according to the Bradford method using bovine serum albumin as protein standard. The albumin-depleted protein solutions were freeze-dried and kept at −20°C until analysis.

2.3. Selenium concentration

Total plasmatic selenium concentration (blood collected in heparin tubes) was determined analytically after the dietary supplementation period. An atomic absorption spectrometer fitted with a graphite furnace (Perkin Elmer, Waltham, MA, USA) was used by following the protocol described by Jacobson and Lockitch [24] but adapted to plasma samples. Selenium concentration was expressed as micromoles of elemental selenium per liter of plasma.

2.4. Two-dimensional gel electrophoresis

Two-dimensional (2D) gel electrophoresis was carried out following the protocol used by Toledo et al. [25] that is based on that described by O'Farrell [26]. Briefly, 300 μg of albumin-depleted plasma protein were resuspended in 50 μl of lysis buffer (9.5 M urea, 2% TritonX-100, 1.6 % ampholytes 4–7 range, 0.4 % ampholytes 3–10 range, and 5 % β-mercaptoethanol), incubated at room temperature for 15 min and loaded onto lab-made first-dimension gels (115-mm-height and 3-mm-internal diameter capillary tubes). A 4.0–7.0 pH gradient was used. Gel prefocusing was carried out at 200 V for 15 min, 300 V for 15 min and 400 V for 15 min. Isoelectric focusing was performed at 400 V for 20 h to complete 8000 Vh. After isoelectric focusing (IEF), the gels were extruded and equilibrated immediately in 2 ml equilibration solution (10% glycerol; 5% β-mercaptoethanol; 2.3 % SDS; 0.0625 M Tris-HCl, pH 6.8) for 10 min. Vertical SDS-PAGE was run with lab-made homogeneous acrylamide gel (11.5 % acrylamide; 180 mm in height and 140 mm wide) at a constant voltage of 50 V during 16 h. Gels were fixed in a 25% methanol–7 % acetic acid solution for 30 min, stained with Coomassie Brilliant Blue R-250 for 12 hours (0.1 % Coomassie blue R250, 25% methanol, 7.5% acetic acid) and destained with a 25% methanol and 7.5% acetic acid solution. Coomassie blue staining is the most reliable quantitative protein staining method, which is widely used in proteomic studies [27]. All chemicals were of analytical grade and were purchased from Sigma (St. Louis, MO, USA). Four independent experiments were carried out for each sample.

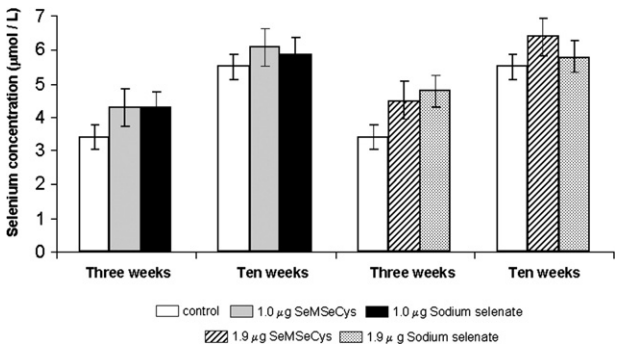

Fig. 1. Plasmatic selenium concentration (μmol/L) in control and experimental groups. Selenium concentration was expressed as elemental selenium per liter plasma.

2.5. Image analysis

Image acquisition was performed with an ImageScanner II device (GE Healthcare, Uppsala, Sweden). Intensity calibration was carried out using an intensity step wedge prior to gel image capture. Image analysis was carried out using the software Total Lab v2.01. Spots were automatically detected and matched. Only the statistically reproducible spots, i.e., the spots that were present in all gels of the same condition, were considered for further analysis. Each spot volume was processed by background subtraction, and spot volumes of all gels were normalized to remove non-expression-related variations in spot volume. The raw quantity of each spot in a gel was divided by the total quantity of all the valid spots in that gel, as recommended in literature [28,29].

2.6. Mass spectrometry analysis

Protein spots were excised directly from the gels and were analysed by MALDI-TOF (Matrix-Assisted-Laser-Desorption-Ionization/Time-Of-Flight) at the University of Colorado Health Sciences Centre. Gel bands were cut into smaller pieces to enhance clean up of bands and trypsin absorption. Bands were manually digested using the standard Proteomics Core protocol [30,31]. Samples were digested overnight at room temperature with modified porcine trypsin. Digest solution was spotted on a MALDI target with alpha-cyano-4-hydroxycinnamic acid for sample cocrystallization. Samples were analysed using MALDI-TOF mass spectrometry in a Voyager DE-STR system (PerSeptive Biosystems, MA, USA). Data was calibrated, deisotoped and centroided, and a peak list was generated. Peak lists were searched using the MASCOT search engine (http://www.matrixscience.com) against the nrNCBI database v20070204/Rodent subset.

3. Results

Total selenium concentration was determined in all the plasma samples in order to investigate the effect of the various treatments on this commonly used selenium status index. Results are shown in Fig. 1. No differences in plasma selenium concentration or body weight were observed between male and female rats (data not shown). Plasmatic selenium concentration was slightly higher in the selenium supplemented animals as compared to the time control

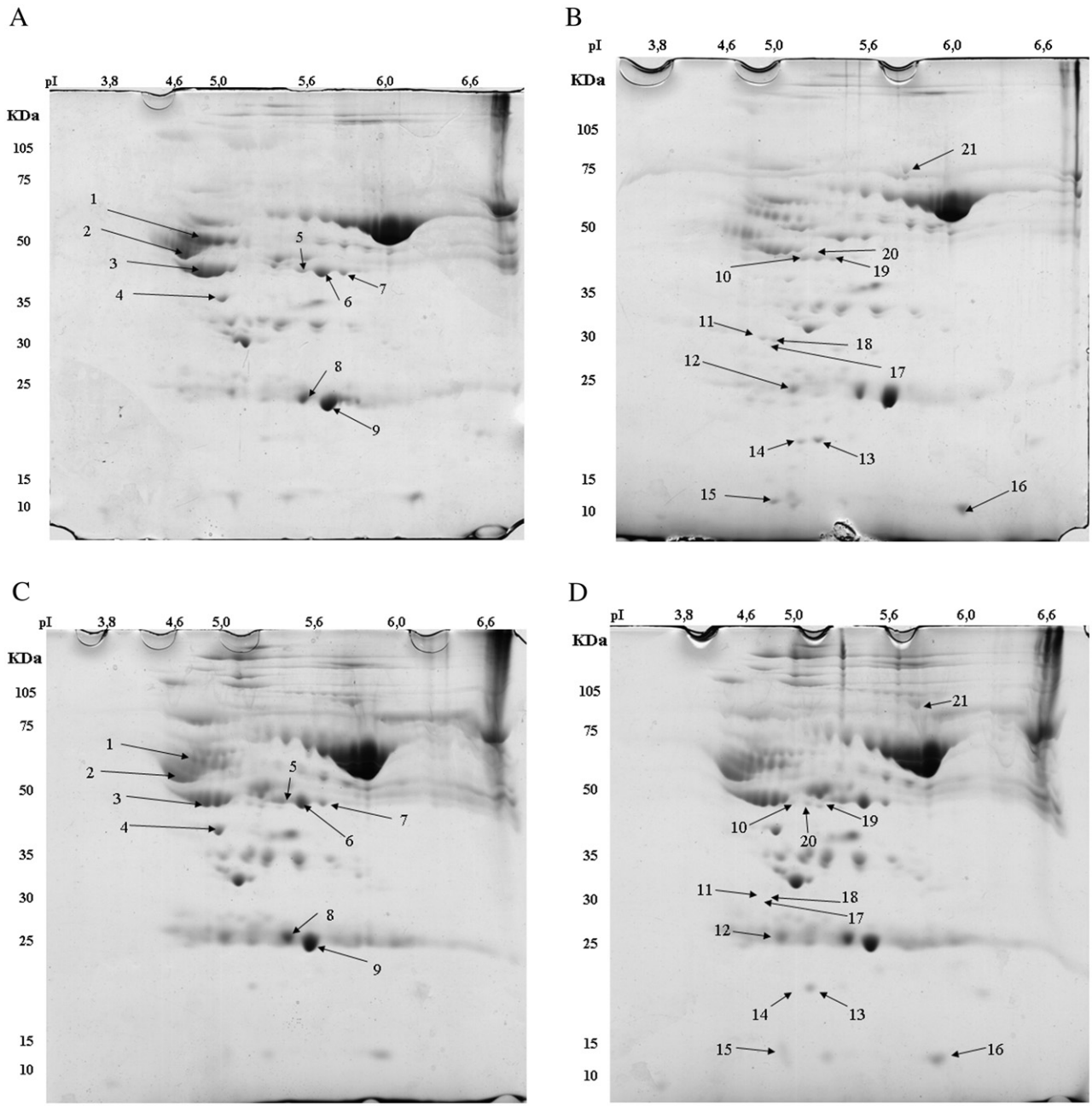

Fig. 2. 2D gel images for the conditions: (A) 0.15 ppm sodium selenate (time control) and three weeks of supplementation; (B) 1.9 ppm selenium as SeMSeCys and three weeks of supplementation; (C) 0.15 ppm selenium as sodium selenate and ten weeks of supplementation (time control); and (D) 1.9 ppm selenium as SeMSeCys and ten weeks of supplementation. Protein spot num are highlighted in the figure. These protein spots were analysed by mass spectrometry in order to determine their identity. For each condition, 2D gels were made in quadruplicate, each gel was densitometrically analysed and spot volume was expressed as the mean±S.D.

groups. However, no statistically significant differences were observed between control and experimental groups at a 95% confidence (P=.327). Additionally, no statistically significant differences in selenium concentration were observed between animals fed with SeMSeCys or sodium selenate at a 95% confidence interval (P=.857).

Fig. 2 shows representative gels for Conditions A (control group for three weeks of dietary supplementation), C (dietary supplementation with 1.9 ppm of selenium as SeMSeCys for 3 weeks), D (control group for ten weeks of dietary supplementation) and F (dietary supplementation with 1.9 ppm of selenium as SeMSeCys for 10 weeks). Results on protein identification are presented in Table 2. Densitometric analysis of the gels corresponding to the experimental conditions and the respective time control groups are presented in Table 3. Reproducibility of quadruplicate densitometric assessments of most of the spots was within the usual range in the literature [28]. Differences between

Table 2
Mass spectrometry analysis of protein spots highlighted in Fig. 2

Spot Nr.	Protein	Sequence coverage (%)	Score	Expect value
1	Onecut1 protein	97	26	3.70E+02
2	Contrapsin-like protease inhibitor (CPi-21)	64	184	6.50E-14
3	α-1-antitrypsin	66	191	1.30E-14
4	Apolipoprotein A-IV	83	338	2.00E-29
5	Fibrinogen	71	220	1.60E-17
6	Fibrinogen	62	174	6.50E-13
7	Fibrinogen	58	135	5.20E-09
8	Apolipoprotein A-I	78	155	5.20E-11
9	Apolipoprotein A-I	83	207	3.30E-16
10	Alpha-1-antitrypsin precursor	63	213	8.20E-17
11	Cyclin H	34	52	9.20E-01
12	Immunoglobulin light chain	64	101	1.30E-05
13	Mouse Apolipo-protein E	46	81	1.20E-03
14	Apolipoprotein E	71	119	2.10E-07
15	Haptoglobin	34	84	6.50E-4
16	Rat Transthyretin	89	106	4.10E-06
17	Apolipoprotein A-IV	33	133	8.20E-09
18	Zinc finger protein 108	27	60	1.60E-01
19	Alpha-1-antitrypsin	66	290	1.60E-24
20	Alpha-1-antitrypsin	56	273	8.20E-23
21	Gelsolin	60	233	8.20E-19

Spot Nr. indicates the number assigned to each spot in Fig. 2; Score and expect value, parameters given by the search engine Mascot in that search (a high score and a low expect value mean a low probability to find that search result randomly, i.e., low probability of that result to be a false positive).

experimental and control groups were determined by using the Student *t* test at a 95% confidence interval.

From the data presented in Table 3, it became evident that some proteins, such as those corresponding to Spots 10, 13, 15, 16, 19 and 20, were more abundant when diets were supplemented with SeMSeCys. Since Spots 10, 11, 13, 14, 15, 17, 18, 19 and 20 were not detected in the control conditions, *P* values could not be calculated, although we considered that these proteins were overexpressed in response to SeMSeCys supplementation.

Besides, we observed statistically significant differences in the normalized volumes of some proteins depending on the experimental treatment. Using 1.0 ppm selenium as SeMSeCys for three weeks (Group B), Spots 10 and 13 (that were expressed only in the experimental condition, see Table 3) showed a higher normalized volume in comparison to the corresponding control (Group A). Proteins with a lower normalized volume in the experimental condition were not considered in this analysis because our aim was to find proteins reflecting SeMSeCys intake. Thus, we focused on proteins showing a higher normalized volume. Using 3 weeks of dietary supplementation with 1.9 ppm SeMSeCys (Group C), Spots 10, 11, 12, 13, 14, 15, 17, 18, 19 and 20 increased their normalized volume. Under dietary supplementation with 1.0 ppm SeMSeCys for 10 weeks, Spots 10, 13, 15, 19 and 20 also showed a higher normalized volume whereas dietary supplementation with 1.9 ppm selenium as SeMSeCys for 10 weeks provoked an increase in the

Table 3
Normalized volume (V_n±S.D.) and *P* value for each experimental condition and control groups

Group	A	B		C		D	E		F	
Spot Nr.	V_n±S.D.	V_n±S.D.	*P*	V_n±S.D.	*P*	V_n±S.D.	V_n±S.D.	*P*	V_n±S.D.	*P*
1	332.19±186.81	278.14±7.02	.722	365.21±60.58	.834	235.36±6.07	294.43±291.66	.802	489.57±55.76	.023*
2	1078.72±219.35	562.16±12.82	.079	400.08±9.66	.048*	443.87±130.50	1059.15±48.12	.025*	465.06±228.15	.919
3	771.72±256.26	812.26±12.81	.844	407.15±25.24	.183	553.05±261.84	667.59±117.23	.629	769.87±15.67	.363
4	62.83±21.66	76.61±10.11	.501	9.53±0.40	.074	91.13±32.77	112.51±27.50	.553	101.26±6.96	.711
5	14.31±12.36	47.52±50.40	.461	0.00±0.00	–	54.75±14.63	8.93±12.63	.079	62.92±16.06	.648
6	245.24±29.47	48.69±27.24	.020*	155.91±40.05	.126	252.21±7.74	81.63±26.53	.013*	257.96±26.44	.796
7	31.80±14.02	0.00±0.00	–	41.64±3.99	.441	4.54±2.07	0.00±0.00	–	7.28±10.29	.747
8	194.10±156.34	168.16±165.33	.887	400.21±43.76	.214	359.05±69.57	324.11±180.69	.822	297.43±8.33	.339
9	843.96±19.04	778.84±158.52	.622	1109.90±335.67	.379	548.86±7.89	655.49±92.88	.247	525.78±47.71	.569
10	0.00±0.00	3.67±5.18	–	45.92±11.58	–	0.00±0.00	8.11±1.46	–	0.00±0.00	–
11	0.00±0.00	0.00±0.00	–	15.66±3.70	–	1.11±1.24	0.00±0.00	–	0.00±0.00	–
12	19.22±27.18	43.15±14.80	.388	126.62±15.58	.04*	161.18±0.24	154.37±20.91	.690	122.34±12.38	.047*
13	0.00± 0.00	10.61±8.86	–	57.77±12.97	–	0.00±0.00	47.08±29.15	–	31.13±5.23	–
14	0.00±0.00	0.00±0.00	–	14.15±11.46	–	0.00±0.00	0.00±0.00	–	0.00±0.00	–
15	0.00±0.00	0.00±0.00	–	18.66±10.39	–	0.00±0.00	28.14±1.07	–	21.71±0.47	–
16	17.19±8.23	33.46±13.55	.495	79.71±33.35	.594	12.94±18.29	31.82±18.66	.414	80.65±2.27	.035*
17	0.00±0.00	0.00±0.00	–	12.22±11.67	–	1.37±1.94	0.00±0.00	–	0.00±0.00	–
18	0.00±0.00	0.00±0.00	–	16.38±2.03	–	0.00±0.00	0.00±0.00	–	0.00±0.00	–
19	0.00±0.00	1.37±1.93		24.91±19.23	–	0.00±0.00	6.72±4.90	–	6.15±3.53	–
20	0.00±0.00	0.00±0.00	–	19.95±2.93	–	0.00±0.00	8.15±11.52	–	1.28±0.26	–
21	0.18±0.26	0.00±0.00	–	0.00±0.00	–	17.34±24.53	0.00±0.00	–	25.65±12.61	.712

When possible, "–" indicates that the *P* value could not be determined because normalized volume in experimental and/or control group was equal to zero; V_n, the average normalized volume of that spot in that experimental group; A, B, C, D, E and F, the treatments as described in Methods and materials section. The mean volume of each spot was calculated from four replicates. Student's *t* test was made considering a 95% confidence interval.

* Statistically significant differences.

normalized volume of Spots 1, 13, 15, 16, 19 and 20 in comparison with the corresponding control group.

4. Discussion

The form of selenium (sodium selenate or SeMSeCys) supplementing the diet did not affect total plasmatic selenium concentration, thus indicating that plasmatic selenium concentration, which does not distinguish between organic and inorganic forms of selenium, is not a suitable index to assess an adequate chemoprotective selenium status.

In this study, Coomassie blue detection was used to determine protein abundance in 2D-electrophoretic gels because of staining reproducibility and because it shows a direct relationship between spot volume and protein abundance [27,32–34]. However, low-abundance proteins cannot be detected by this technique. This work focused on the detection of differences in protein abundance in rat blood plasma due to SeMSeCys intake and not on the detection of specific proteins. Accordingly, Coomassie blue detection is adequate as a first approach to detect differences in high-abundance proteins.

As shown in Fig. 2 and Table 3, some plasma proteins could be detected only under diet supplementation with SeMSeCys. Some of those proteins showed a higher normalized volume only in the 3-week treatment, but it decreased when supplementation lasted longer (10 weeks). Other group of proteins, including apolipoprotein E (13), haptoglobin (15), transthyretin (16) and α-1-antitrypsin (19), showed a higher normalized volume both at three and 10 weeks of diet supplementation, depending on the dose of SeMSeCys. Fig. 3 shows normalized volume profiles of these proteins and its dependence on selenium dose.

In order to determine which proteins may be potential biomarkers for SeMSeCys intake, the following aspects were considered. Proteins with an increased normalized volume only after 3 weeks but not after 10 weeks of diet supplementation with SeMSeCys were discarded as possible biomarkers because they were not expressed in direct relation with the experimental conditions in the study. This was the case of α-1-antitrypsin (spot 19). Its higher normalized volume observed after three weeks of diet supplementation with 1.9 ppm selenium, as SeMSeCys may be related either to physiologic conditions other than SeMSeCys supplementation or may be involved in growth and development processes occurring in the experimental young animals. Consequently, this protein would not reflect the effect of diet supplementation with organic forms of selenium on the blood plasma proteome.

In the cases of apolipoprotein E (spot 13) and haptoglobin (spot 15), we observed an increase in the normalized volumes in proportion to the dose of SeMSeCys given during three weeks of diet supplementation. However, after 10 weeks of supplementation with 1.0 ppm selenium (as SeMSeCys), a maximum normalized volume was observed whereas supplementation with 1.9 ppm selenium (as SeMSeCys) for the same period resulted in a decrease in the normalized volume. This observation suggests that the increases in the normalized volumes of

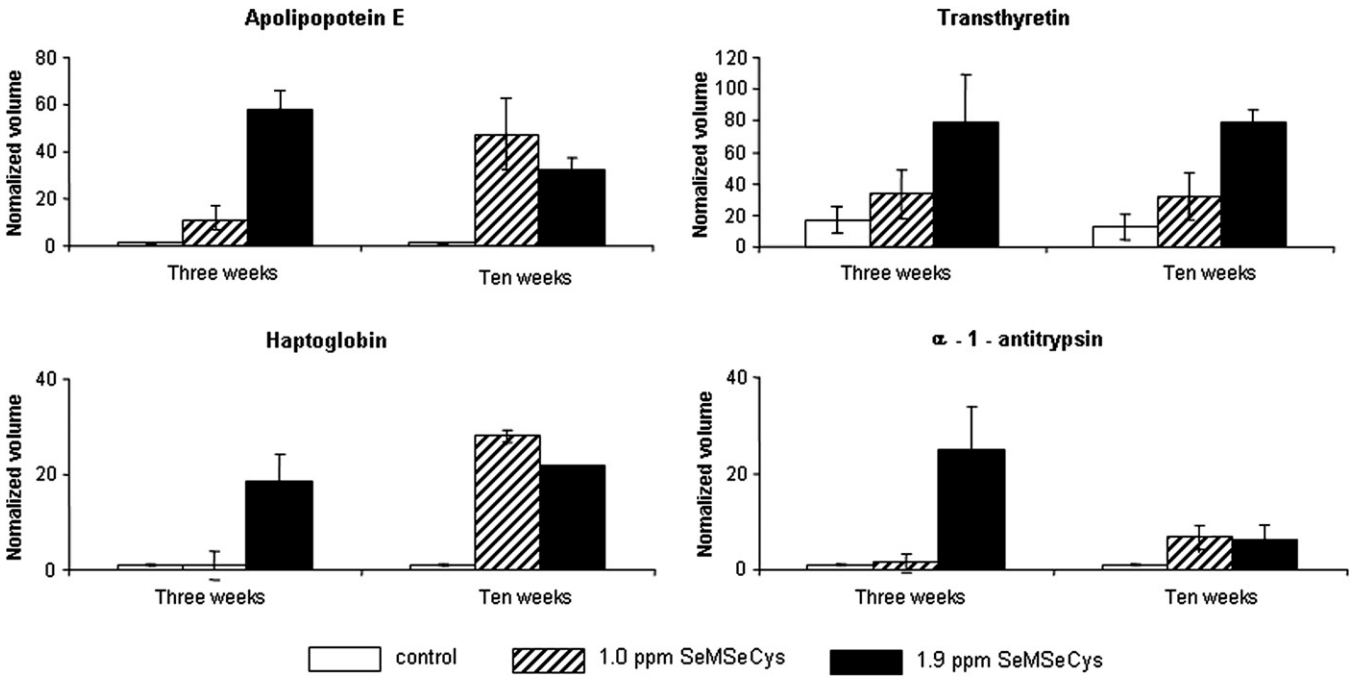

Fig. 3. Normalized volume (arbitrary units) profiles and its dependence with SeMSeCys dose for the proteins apolipoprotein E (Spot 13), transthyretin (Spot 16), haptoglobin (Spot 15), and α-1-antitrypsin (Spot 19).

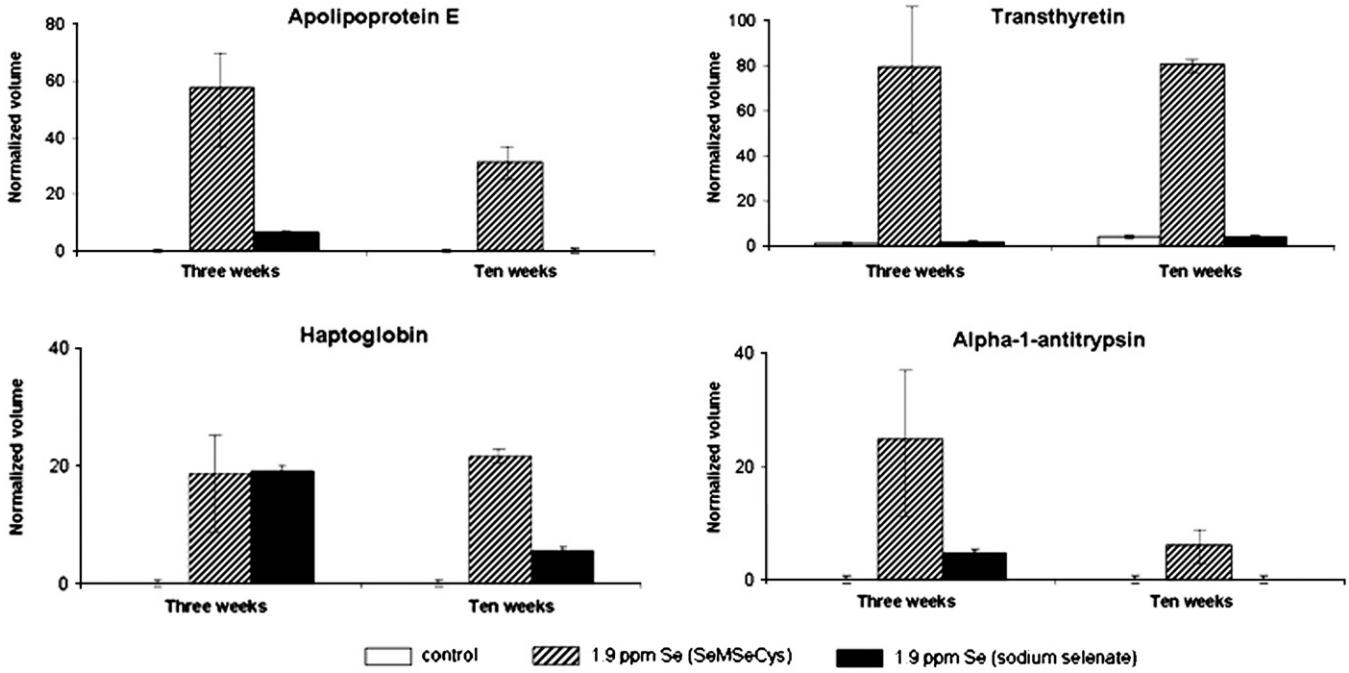

Fig. 4. Normalized volume of apolipoprotein E (Spot 13), transthyretin (Spot 16), haptoglobin (Spot 15) and α-1-antitrypsin (Spot 19), using 1.9 ppm Se as SeMSeCys and different supplementation periods, compared to the same dose of sodium selenate.

both proteins do not show a direct association with the experimental factors in the study: dose of SeMSeCys and the extent of the dietary supplementation period. However, both proteins were more abundant in all the experimental groups supplemented with SeMSeCys, and so they would be potential biomarkers of metabolic status of organic forms of selenium. Saturation behaviour was not observed for these proteins, that is, their normalized volumes did not reach a maximum and constant value. That behaviour is observed in metabolic pathway saturation.

A statistically significant increment in the normalized volume of transthyretin (spot number 16) was observed only after ten weeks of diet supplementation with 1.9 ppm selenium (as SeMSeCys). After three weeks of supplementation with the same SeMSeCys dose, a normalized volume increment was observed but it was not statistically significant probably due to the high standard deviation in the experiments (coefficient of variation equal to 57.5 %). Normalized volumes of transthyretin were about 32 arbitrary units (AU) after 3 or 10 weeks of supplementation with a 1.0-ppm dose, whereas they were as high as 80 AU when the dose given in the same periods was raised to 1.9 ppm. Thus, the relative abundance of this protein seemed to be proportionally affected by the dose of SeMSeCys but unaffected by the extent of the supplementation period. Accordingly, transthyretin is a potential biomarker for SeMSeCys intake.

Relative abundances of apolipoprotein E, transthyretin, haptoglobin and α-1-antitrypsin in the selenium type controls were analysed (Experimental Groups G, H, I and J). In these studies, 2D gel electrophoresis analysis was carried out in order to determine which proteins were overexpressed in response to SeMSeCys supplementation but not in response to sodium selenate supplementation. Again, the spots corresponding to those proteins were matched and quantified, and the normalized volumes were compared to the corresponding time controls (Experimental Groups A and D). Fig. 4 shows the normalized volumes of these plasma proteins after diet supplementation with 1.9 ppm selenium either as sodium selenate or as SeMSeCys and in rats of the time control groups. No statistically significant differences were observed in the normalized volumes of haptoglobin and α-1-antitrypsin after supplementation with the same dose of SeMSeCys or sodium selenate (P=.337 at a 95% confidence interval for both proteins). Then, these proteins seem to reflect selenium intake without distinguishing between organic and inorganic forms of selenium. No statistically significant differences were observed in apolipoprotein E and transthyretin expression in the blood plasma of rats fed a sodium selenate supplemented diet as compared to the time controls (P=.422 for apolipoprotein E and P=.917 for transthyretin, at a 95% confidence interval). Besides, statistically significant differences were indeed observed between normalized volumes of these proteins in rats fed a 1.9 ppm selenium (as SeMSeCys) supplemented diet as compared to rats fed the same dose of sodium selenate (P=.044 for apolipoprotein E and P=.0005 for transthyretin, at a 95% confidence interval). Thus, the relative abundances of these two proteins seem to be related to the form of selenium (organic or inorganic) supplementing the diet. Abundance is significantly higher when a chemoprotective form of selenium is used.

Apolipoprotein E is a secreted protein present in blood plasma. It mediates binding, internalization and catabolism of lipoprotein particles. This protein serves as ligand for the low-density lipoprotein receptor and for the specific apolipoprotein E receptor in liver tissues. It has been reported that selenium deficiency results in increased levels of apolipoprotein E in rat plasma although the mechanism by which selenium deficiency affects lipoprotein metabolism is poorly understood [35]. Housekeeping selenoproteins, which would have a role in regulating lipoprotein biosynthesis and metabolism, have been involved [36]. The inverse relationship between selenium deficiency and apolipoprotein E concentration in blood plasma is in contradiction with the observations in this study, probably due to the different nature of selenium used by those authors [35,36].

Transthyretin is a hormone-binding protein, rich in aromatic amino acids, that transports thyroxine from the blood stream to the brain. It is synthesized in liver and in the choroid plexus [37]. This protein is involved in the thyroid hormone metabolism and selenium exerts a major control function on the thyroid hormone homeostasis [38]. This control is performed through the antioxidant activity of several selenoenzymes that are synthesized in thyroid gland [39]. Those selenoenzymes are produced only if the selenoaminoacid SeCys is available and SeMSeCys would be the source of bioavailable selenium. Then, transthyretin would indirectly reflect the overexpression of selenoenzymes in the thyroid gland. This protein would not be overexpressed when inorganic forms of selenium supplement the diet because this form of selenium would be less bioavailable.

On the other hand, since SeMSeCys is known as having a chemoprotective effect, it would be expected that proteins considered as chemoprotective enzymes would increase their expression level when SeMSeCys is supplementing the diet. Among the chemoprotective enzymes are glutathione S-transferase isoforms, glucuronosyltransferases, glutathione peroxidase 1, thioredoxin reductase, microsomal epoxide hydrolase and NAD(P)H-quinone oxidoreductase 1. El-Sayed et al. [40] studied the effect of different L-selenocysteine derivatives on mRNA levels of these chemoprotective enzymes in liver. The authors found that each selenocysteine prodrug elicited a unique pattern of mRNA response. In a subsequent study, El Sayed et al. [41] found that SeMSeCys enhanced glutathione S-transferase activity and increased the levels of its mRNA. However, in the present study, no statistically significant differences were detected regarding any chemoprotective enzyme, probably due to the very low concentration of these enzymes in blood and the low sensitivity of the protein detection method. Another possible explanation would be the localization of the chemoprotective enzymes. El-Sayed et al. [40,41] assayed liver extracts, where the above-mentioned chemoprotective enzymes are synthesized and are present in relatively high concentrations. In the present study, blood plasma proteome was investigated in order to identify proteins with potential to be used in a non-invasive way as selenium status biomarkers. If a blood plasma protein is found to increase its expression level in response to dietary supplementation with a chemoprotective form of selenium, as SeMSeCys, then that protein could be used as biomarker in a noninvasive assay with a prospective use in humans.

Despite that apolipoprotein E and transthyretin are not considered chemoprotective proteins; we can speculate that they have potential to be used as plasmatic biomarkers of organic selenium forms. However, studies on their expression in liver and other tissues should be further investigated.

In summary, a proteomic approach allowed for detection of protein expression differences in rat plasma in response to SeMSeCys dietary supplementation. Statistically significant differences in the normalized volume of some proteins were observed in all experimental groups. Apolipoprotein E, haptoglobin and α-1-antitrypsin showed a normalized volume increase that was proportional to the time of supplementation when a 1.0-ppm SeMSeCys dose was used. However, this tendency changed at the higher dose of 1.9 ppm SeMSeCys. Apolipoprotein E showed a direct relationship between normalized volume and extent of supplementation period, but not with the dose of SeMSeCys. Transthyretin showed a higher normalized volume that was proportional to SeMSeCys dose but not to the extent of the supplementation period, probably due to saturation of the metabolic pathway in a time period shorter than three weeks. Then, the response to SeMSeCys dietary supplementation involving transthyretin would be faster than the one involving apolipoprotein E. Finally, overexpression of apolipoprotein E and transthyretin occurred only in response to a chemoprotective form of selenium, and then we propose that both proteins have potential as biomarkers for bioactive selenium status.

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