Comparison of Sodium Selenite and Selenium-Enriched Spirulina Supplementation Effects After Selenium Deficiency on Growth, Tissue Selenium Concentrations, Antioxidant Activities, and Selenoprotein Expression in Rats

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Research Article

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Abstract

Selenium contributes to physiological functions through its incorporation into selenoproteins. It is involved in oxidative stress defense. A selenium deficiency results in the onset or aggravation of pathologies. Following a deficiency, the repletion of selenium leads to a selenoprotein expression hierarchy still misunderstood. Moreover, spirulina, a microalgae, exhibits antioxidant properties and can be enriched in selenium during its cultivation. Our objective was to determine the effects of a sodium selenite or selenium-enriched spirulina supplementation. Thirty-two female wistar rats were fed for 12 weeks with a selenium-deficient diet. After 8 weeks, rats were divided into 4 groups of 8 rats and were fed with water, sodium selenite (20μg Se/kg body weight), spirulina (3 g/kg bw) or selenium-enriched spirulina (20μg Se/kg bw + 3g spirulina/kg bw). In parallel, another group of 8 rats were fed with normal diet during 12 weeks. Selenium concentration and antioxidant enzyme activities (GPx, SOD, CAT) were measured in plasma, urines, liver, brain, kidney, heart and soleus. Expression of GPx (1, 3), Sel (P, S, T, W), SEPHS2, TrxR1, ApoER2 and Megalin were quantified in liver, kidney, brain and heart. Our results showed that a selenium deficiency leads to a growth retardation, reversed by selenium supplementation. All tissues displayed a decrease in selenium concentration following deficiency. Brain seemed protected. Our results demonstrated a hierarchy in selenium distribution and selenoprotein expression depending on selenium supplementation form. A supplementation of sodium selenite improved GPx activities and selenoprotein expression while a selenium-enriched spirulina was more effective to restore selenium concentration.

Introduction

Selenium (Se) is an essential trace element for humans and animals' life. It is provided by diet in organic (selenomethionine, selenocystein) or inorganic forms (selenite, selenate…). Physiological functions of Se are mainly linked to its incorporation in selenoproteins such as Glutathion Peroxidase or thioredoxin reductase [1, 2]. Indeed, selenoproteins are involved in reproduction, growth, muscle metabolism, immune system or fertility [3–5]. For example, selenoprotein W (SelW) is a ubiquitous protein mainly expressed in brain and muscles it displays antioxidant properties in various animal models using GSH-dependent and Thioredoxin motif CXXU pathways [6, 7]. Selenoprotein P (SelP) is mostly expressed in the liver and represents the main blood selenium carrier allowing to maintain adequate selenium concentration in the organism [8]. Selenoprotein S, for its part, is involved in ER-stress defense pathway [9]. In general, selenium is predominantly involved in the response against oxidative stress due to the high reactivity of the selenocysteine residue (that substitutes a cysteine in selenoproteins). Furthermore, a severe selenium deficiency can be responsible for development of pathologies such as myocardial dysfunctions (Keshan Disease) [10–12] or kidney disorders [13, 14]. A decrease in antioxidant activities has also been observed in selenium-deficiency animals [15–18]. Many studies have shown that a selenium deficiency also leads to the establishment of a selenoprotein expression hierarchy [19–21]. Nevertheless, the restoration of Se levels and tissue redistribution after a supplementation in selenium-deficient animals are still widely misunderstood and requires further investigations to be elucidated.

Spirulina is a blue-green algae known for its nutritional properties. It was described in 1992 as the best food for future by WHO. It is widely used as a dietary complement for its high content in proteins, vitamins, β-

caroten and pigments such as phycocyanin [22]. Spirulina also exhibits anti-inflammatory [23, 24], anti-cancer [25] and antioxidant properties [26]. Indeed, in vivo and in vitro models show oxidative stress marker reduction and antioxidant enzyme activities improvement after a spirulina supplementation [27, 28]. Moreover, during its culture, spirulina can be enriched with elements such as selenium and incorporate selenium into organic molecules (SeMet and SeCys).

In this context, the aim of this study was:

- To determine how selenium is distributed between different organs after selenium deficiency and repletion.
- To investigate the selenoprotein expression hierarchy and antioxidant enzyme activities in the same conditions.

In order to achieve these goals, selenium deficient rats received either Na_2SeO_3 or Se-enriched spirulina. After a growth analysis, Se concentration, antioxidant enzyme activities and gene expression of selenoproteins were analysed on a panel of tissues related to selenium physiology.

Materials And Methods

Spirulina platensis powder

The *Spirulina* strain used in this study was *Spirulina platensis.* Production and conditioning of spirulina and Se-enriched spirulina was carried out by TAM company (Plougastel, France). Spirulina devoid of selenium was dried and transformed into powder. At the same time, a spirulina enriched with selenium was produced and processed identically. In the case of Selenium-enriched *Spirulina*, the final selenium concentration was 55 µg Se/g of S*pirulina platensis* (dry weight).

Ethical approval

This study was performed in accordance with the recommendations of the European Community directive 2010/63/EU and was approved by the regional ethical committee (CEFEA: Comité d'Ethique Finistérien en Expérimentation Animale" – departmental agreement No. B29-019-08) and the French Ministry in charge of animal experimentation (protocol No. 18325_2018123119211520). Authors understand the journal ethical principles and this study complies with this animal ethics requirements. In particular, pain and suffering were minimized during the entire experiment.

Experimental protocol

Animals

40 three-week-old female Wistar rats (Janvier, SAS-Le Genest St Isle) with an average weight of 85.7 ± 1.5 g were included in this study. Rats were raised under a 12 hours light-12 hours dark cycle at 21°C. Food and water were given *ad libitum*. Rats were allowed to adapt to environmental conditions for one week before experiments.

Diet and supplementation

A group of 8 rats were fed with a standard diet for 12 weeks (containing 0.3 mg Se/kg food, ref n°3430, Mouse and Rat maintenance, Kliba Nafag, Switzerland ; Group C, C for control without deficiency. The others 32 rats were fed with a diet devoid of selenium (ref n°U8959P v.0170, SAFE, France) for 12 weeks. After 8 weeks, these 32 rats were randomly assigned to one of the four following experimental groups (8 rats/ group) and received during 4 weeks:

- only water (D group, “D” for deficient);
- sodium selenite (Na_2SeO_3, 71950, Sigma Aldrich) dissolved in water in order to reach 20 µg Se/kg body weight (bw) per day (Se group);
- 3 g /kg bw /day of *spirulina* (Spi group)
- 3g /kg bw /day of Se enriched *spirulina*, bringing a dose of 20 µg selenium /kg bw /day (SeSP group).

The 32 animals were fed with the selenium-deficient diet during the supplementation period (Table 1). All rats were weighted weekly. Food and drink intakes were measured twice a week during all the experiment.

Table 1
Experimental Protocol

Group	**n**	**Diet (from W0 to W12)**	**Supplementation (from W8 to W12)**
C	8	Normal	None
D	8	Se defficient	None
Se	8	Se defficient	20 µg /kg bw /day of Se (with Na_2SeO_3 in water)
Spi	8	Se defficient	3 g /kg bw /day of spirulina (in water)
SeSp	8	Se defficient	3 g /kg bw /day of Se enriched spirulina (in water) bringing 20 µg /kg bw /day of Se

Analytical procedure

At week 12, animals were anesthetized by ketamine (100 mg/kg) and xylazine (10 mg/kg) injection. Animals were euthanized by exsanguination (intracardiac puncture). Urine and organ samples were collected at the animal’s death and immediately frozen in liquid N_2. Frozen samples were kept at -80°C until analyses.

Antioxidant enzyme activities

70 mg samples of frozen diaphragm, long peroneal, gastrocnemius, extensor digitorum longus (EDL), soleus, heart, kidney and liver and were homogenized with a Polytron homogenizer in an extraction buffer

(75 mM TRIS and 5 mM EDTA, pH 7.4, 4°C). After a centrifugation at 12,000 g for 10 min at 4°C, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were determined on the resulting supernatant

GPx activity was measured at 340 nm with an indirect method adapted from Ross *et al.* (2001) by Farhat *et al.* (2015) [29, 30]. Briefly, the activity was determined from the decrease of NADPH induced by a coupled reaction with glutathione reductase. GPx activity was expressed in nmol NADPH oxidized/min/g wet tissue.

SOD activity was assessed at 480 nm using an indirect method that inhibits the adrenaline to adenochrome reaction with the xanthine/ hypoxanthine reaction as a superoxide anion producer (Misra and Fridovich, 1972). One unit (U) of SOD activity corresponds to the amount of sample needed to cause 50% inhibition relative to the control without tissue. SOD activity was expressed in U/mg wet tissue.

Catalase activity was determined at 240 nm through its capacity to transform hydrogen peroxide (H_2O_2) into water and oxygen (Beers and Sizer, 1952). The adding of 200 mM H_2O_2 initiated the reaction. CAT activity was expressed in nmol H_2O_2/min/g wet tissue.

Measurement of selenium concentrations in tissues

100 µL of plasma or urine samples, were weighed in PTFE vials. The samples were dissolved using 2 mL of distilled 14N HNO_3, and evaporated overnight at 90°C to dryness. 1 mL of 2.5% HNO_3 was then added to the samples for ICP-MS measurements.

100 mg of tissues (diaphragm, long peroneal, gastrocnemius, extensor digitorum longus, soleus, heart, kidney and liver) were weighed in PTFE vials. The samples were dissolved using 2 mL of distilled 14N HNO_3 and 0.5 mL H_2O_2, and evaporated overnight (90–100°C) to dryness. 4 mL of 2% HNO_3 + Indium 1 ppb was added for ICP-MS measurements. Samples were then placed on a hot plate (60°C) overnight. The concentrations of selenium in the sample solutions were measured with a HR-ICP-MS Element XR (Thermofisher Scientific) at “Pôle de Spectrométrie Océan (PSO) (IUEM/Ifremer, Brest, France)” using indium as an internal standard for a drift signal correction. Selenium was measured at medium resolution to compensate spectral interferences forming in the argon plasma. Concentrations were calibrated using external calibration standards prepared for the measurements. The detection limit was ~ 3 ng/g. Two procedural blanks were also processed following the above protocol and analyzed with the samples.

Selenoprotein expression

Total RNA was isolated from liver, brain, kidney and heart tissues using the Nucleospin RNA Set (740406.50, Macherey-Nagel, France) according to a manufacturer’s protocol adapted for total blood. Briefly, an enzymatic lysis was performed with 200 µL of Lysis Buffer and 5 µL of Proteinase K during15 min at room temperature. Then, 200 µL of 70% ethanol was added and lysate was transferred into a Nucleospin column. After centrifugation at 11000 g for 30 s, 350 µL of Membrane Desalting Buffer was added onto the column and centrifuged identically. The following RNA extraction steps, which included a DNase treatment, were performed according to the kit procedure. At the end, RNA was eluted with 40 µL of DNase/RNase-free water

and stored at -80°C. RNA concentrations were measured with a SimpliNano™ spectrophotometer (29-0617-12, GE Healthcare Life Sciences) and their purity was assessed using OD_{260}/OD_{280} ratio. Their integrity was also checked by an electrophoresis on a 1.5% agarose gel with ethidium bromide.

GPx1, GPx3, SEPHS2, SelW, SelS, SelP, SelT, ApoER2, Megalin and TrxR1 were quantified by RT-PCR. Total RNA (1000ng) was reverse transcribed with the qScript cDNA synthesis kit (733–1174, QUANTA BioSciences, VWR, France) containing a mix of oligo (dT) and random primers, dNTPs, Mg^{2+} and the Reverse Transcriptase. All cDNAs were then diluted 10-fold for PCR experiments, which were realized with a 7500 Fast Real-Time PCR (Applied Biosystems, Thermo Fisher Scientific, France). Target genes were amplified and quantified by SYBR® green incorporation (EurobioGreen® Mix qPCR 2x Lo-Rox, Eurobio Ingen, Courtaboeuf, France) with the primers given in Table 2.

Table 2
Primer sequences used for Real-Time RT-PCR analysis

Target gene	Abbreviation	Primer sequence (5' to 3')	Accession number	Data base
Glutathion Peroxidase 3	*Gpx3*	(F) CAAGAAGAACTTGGCCCATTC	BC062227	GenBank
		(R) GCTGGAAATTAGGCACAAAGC		
Glutathion Peroxidase 1	*Gpx1*	(F) TGCAATCAGTTCGGACATCAG	NM_030826.4	GenBank
		(R) TTCACCTCGCACTTCTCAAAC		
Glyceraldehyde 3-phosphate deshydrogenase	*Gapdh*	(F) GTATCCGTTGTGGATCTGACA	P04797	GenPept
		(R) CTGCTTCACCACCTTCTTGAT		
Apolipoprotein E receptor 2	*ApoER2*	(F) CGACTGCAAGGACAAGTCTGA	D3ZE75	Uniprot
		(R) CCTGGTTGCACCGTTTGATTG		
Megalin	*Megalin*	(F) CGCCATTTGGAGGAGAATGCT	A0A0G2K9W7	Uniprot
		(R) CCCTGTCGGTTTTCACACTTC		
Selenoprotein P	*SelP*	(F) GCAGGTGTCAGATCACATTGC	A0A0G2JU99	Uniprot
		(R) GAGTAGGGCAAACCAAGGTGA		
Selenoprotein S	*SelS*	(F) CGACAAGAGGCTTTAGCAGCT	A0A0G2JSM1	Uniprot
		(R) CTTCTGCCTTCTTGCATGCTG		
Selenoprotein T	*SelT*	(F) CATAGCCCTACCTATCAGCAC	F8WFN1	Uniprot
		(R) GCTTGCTGTCTTCAGTACAGG		
Selenoprotein W	*SelW*	(F) CAGGTCACCGGGTTCTTTGAA	A0A0G2JY20	Uniprot
		(R) CACCAGTTTCCGGAACTTGCT		
Selenophosphate synthetase 2	*SEPHS2*	(F) CCACCAATGGCTGGATAATCC	XM_032892581.1	NCBI
		(R) CAGCAGTCCTGTTCAGAGTAG		
Thioredoxin	*TrxR1*	(F) CATGCCGACCTTCCAGTTCTA	R4GNK3	Uniprot

reductase 1	(R) CTCCGTAATAGTGGCTTCGAG

The cycling conditions consisted of a denaturing step at 95°C for 2min, followed by 40–45 cycles of amplification (denaturation: 95°C for 5s; annealing/extension step: 60°C for 30s). Finally, a melting curve program was carried out from 60°C to 95°C with a heating rate of 0.1°C/s, showing a single product with a specific melting temperature for each gene and sample evaluated.

To obtain standard curves, all target genes were first amplified from a pool of RT products prepared with all rat samples. PCR products obtained were purified after electrophoretic separation on a 1.5% agarose gel using the Nucleospin gel and PCR Clean-Up kit (Macherey-Nagel). PCR products were then quantified using a SimpliNano™ spectrophotometer before proceeding to a serial dilution from 10pg/μL to 0.001fg/μL. These seven-point standard curves were used to determine the PCR efficiency of each primer pair.

The calculation of absolute mRNA level of a specific target gene was based on the PCR efficiency value (E) and the Threshold Cycle deviation (ΔCT) of an unknown cDNA versus a control one (here, a pool of blood cDNA) according to the equation proposed by Pfaffl *et al.*, 2001 [31]: absolute mRNA level of a target gene = $E_{target}^{\Delta CT(control-sample)}$. For one gene, four runs were required to quantify the mRNA levels in all samples. Each run included no-template controls and triplicates of the control cDNA. Inter-assays variations were found to be < 1.0%. To account for variations due to mRNA extraction and reverse-transcription reaction, absolute mRNA levels obtained were corrected by 18s rRNA levels, used as a housekeeping gene. Thus, relative mRNA levels were expressed in arbitrary unit as ratios target genes/18S rRNA.

Statistics

All results were expressed as mean ± Standard error of mean (SEM). Statistics were performed using GraphPad Prism v9.0.2 software. Normality was tested using the Shapiro-Wilk test. Adapted tests were then performed (ANOVA or student test). ANOVA was followed by Tukey's post-hoc test. A p-value < 0.05 was considered significant.

Results

Table 3
Animal weight (in g) at the end of the deficiency period (week 7) and during the supplementation period (weeks 8 to 12). Results are presented as mean ± SEM (n = 8 for each group). C, Control Group; D, deficient group; Se, selenium supplemented group; Spi, Spirulina supplemented group; SeSP, selenium-enriched Spirulina supplemented group. For deficiency part, different letters indicate a significant difference (p-value < 0.05). For supplementations part, * indicates a significant difference with C group (p-value < 0.05).

Group	Week					
	7	8	9	10	11	12
C	215,37 [a] ± 5.62	215,87 ± 6.27	223,25 ± 6.10	230,75 ± 6.28	232,25 ± 5.95	235,25 ± 6.05
D	191,56 [b] ± 2.96	192,62 * ± 4.09	198,00 * ± 3.92	207,00 * ± 4.52	209,12 * ± 4.86	208,37 * ± 3.76
Se		191,87 ± 8.31	198,12 ± 8.11	207,00 ± 7.94	211,37 ± 8.09	206,25 * ± 6.17
Spi		198,87 ± 5.50	204,50 ± 4.85	210,87 ± 5.11	216,50 ± 5.26	219,25 ± 5.69
SeSP		195,50 ± 6.32	203,25 ± 6.49	213,00 ± 6.42	213,12 ± 5.61	217,75 ± 5.65

Growth analysis

Mean rat weight was 85,7 ± 1,6 g at the beginning of the experiment (week 0). Eight weeks after (at week 7), rats fed with selenium deficient diet showed a significant decrease of weight compared to the control group fed with standard diet (Mean weight : 191,6 ± 3,0 g compared to 215,4 ± 5,6 g). As shown in table 3, this difference continued until the 12th weeks of the experiment in the case of the D group. During the 4 weeks of supplementation, rat weights increased and no significant difference was observed with the C group except for Se rats.

Selenium concentration in tissues

The plasmatic selenium concentration in Se (493.6 ± 15.6 ppb) and SeSP (535.4 ± 14.2 ppb) groups are not different from the C group (532.2 ± 18.7 ppb) while D (164.5 ± 7.6 ppb) and Spi (155.7 ± 8.3 ppb) groups showed important reductions of [Se] (Fig. 1A). A similar profile is observed for urinary selenium concentration (Fig. 1B) with no significant difference between C (451.4 ± 71.9 ppb), Se (259.4 ± 37.1 ppb) and SeSP (316.7 ± 65.7 ppb) groups while in D and Spi groups, urinary selenium concentration was below the detection limit (Fig. 1B). Concerning liver selenium concentration (Fig. 1C), C (1.8 ± 0.1 ppm) and SeSP (1.7 ± 0.2 ppm) groups displayed no significant difference. However, Se group (1.2 ± 0.1 ppm) showed a significant reduction compared to C and SeSP groups. Once again, D (0.1 ± 0.003 ppm) and Spi group (0.1 ±

0.01 ppm) had a low selenium concentration compared to the other groups. No significant difference was observed in any group in selenium brain concentration (Fig. 1D). In kidney (Fig. 1E), no significant difference appeared between C (1659.0 ± 96.9 ppb), Se (1387.0 ± 94.7 ppb) and SeSP group (1856.0 ± 137.1 ppb). However, Se group displayed a lower selenium concentration compared to SeSP. D (664.7 ± 41.0 ppb) and Spi (661.7 ±18.4 pp) groups showed a lower kidney selenium concentration compared to C, Se and SeSP groups. In heart (Fig. 1F), selenium concentration was higher in C group (447.3 ± 17.8 ppb) compared to Se (325.2 ± 13.4 ppb) and SeSP (377.2 ± 24.7 ppb). No significant difference was observed between Se and SeSP. D (103.7 ± 11.0 ppb) and Spi (115.8 ± 7.3 ppb) heart selenium concentration was also significantly reduced compared to three other groups. Soleus selenium concentration (Fig. 1G) displayed no significant difference between C (317.7 ± 20.0 ppb) and SeSP (310.5 ± 22.3 ppb) while Se (172.8 ± 12.0 ppb) selenium concentration was lower than these two groups. D (64.33 ± 4.8 ppb) and Spi (63.0 ± 3.0 ppb) groups showed reduced selenium concentration compared to C, Se and SeSP groups.

Antioxidant enzyme activities

Glutathione Peroxidase activities (GPx)

In diaphragm (Fig. 2A), C group (1.82 ± 0.16 $NADP^+$/min/mg of tissue) displayed higher GPx activities than all other groups. No significant difference appeared between Se (1.34 ± 0.12 $NADP^+$/min/mg of tissue) and SeSP (1.41 ± 0.06 $NADP^+$/min/mg of tissue) and between D (0.30 ± 0.02 $NADP^+$/min/mg of tissue) and Spi (0.30 ± 0.02 $NADP^+$/min/mg of tissue). However, Se and SeSP GPx activities was higher than D and Spi groups. Long peroneal GPx activities (Fig. 2B) displayed the same profile with higher GPx activities in C group (0.72 ± 0.03 $NADP^+$/min/mg of tissue) compared to others. Similarly, Se (0.40 ± 0.02 $NADP^+$/min/mg of tissue) and SeSP (0.46 ± 0.02 $NADP^+$/min/mg of tissue) displayed no significant difference but were higher than D (0.05 ± 0.01 $NADP^+$/min/mg of tissue) and Spi (0.05 ± 0.01 $NADP^+$/min/mg of tissue) groups. This profile was also found in gastrocnemius (Fig. 2C) and EDL (Fig. 2D) GPx activities. In kidney (Fig. 2E), no significant difference appeared between C (2.56 ± 0.21 $NADP^+$/min/mg of tissue), Se (2.83 ± 0.14 $NADP^+$/min/mg of tissue) and SeSP (3.14 ± 0.15 $NADP^+$/min/mg of tissue) groups while D (0.61 ± 0.04 $NADP^+$/min/mg of tissue) and Spi (0.64 ± 0.03 $NADP^+$/min/mg of tissue) showed a significant reduction in GPx activities compared to these groups. In liver (Fig. 2F), no difference was observed between C (17.61 ± 2.01 $NADP^+$/min/mg of tissue), Se (14.40 ± 1.28 $NADP^+$/min/mg of tissue) and SeSP (15.62 ± 1.35 $NADP^+$/min/mg of tissue) as shown in kidney. Nevertheless, in heart (Fig. 2G), Se GPx activities (1.30 ± 0.06 $NADP^+$/min/mg of tissue) was increased compared to SeSP (0.94 ± 0.07 $NADP^+$/min/mg of tissue) but stayed still lower than C group (1.61 ± 0.06 $NADP^+$/min/mg of tissue). However, in soleus (Fig. 2H), SeSP (1.74 ± 0.18 $NADP^+$/min/mg of tissue) displayed a higher GPx activities than Se (1.16 ± 0.10 $NADP^+$/min/mg of tissue) but remained lower than C group (2.42 ± 0.11 $NADP^+$/min/mg of tissue).

Superoxyde dismutase activities (SOD)

Diaphragm SOD activities (Fig. 3A) displayed no significant difference between C (0.27 ± 0.05 U/mg of tissue), D (0.18 ± 0.05 U/mg of tissue) and SeSP (0.30 ± 0.06 U/mg of tissue) groups. However, Se (0.45 ±

0.04 U/mg of tissue) and Spi (0.44 ± 0.05 U/mg of tissue) SOD activities are significantly higher than D group. No significant difference was observed between all groups in long peroneal (Fig. 3B), gastrocnemius (Fig. 3C) and EDL (Fig. 3D) SOD activities. Kidney SOD activities (Fig. 3E) displayed no significant difference between C (1.78 ± 0.45 U/mg of tissue), D (2.07 ± 0.52 U/mg of tissue), Se (1.77 ± 0.69 U/mg of tissue) and Spi (2.78 ± 0.26 U/mg of tissue) groups. SeSP group (0.73 ± 0.25 U/mg of tissue) showed a significant reduction compared to Spi group but remained not significantly different with C, D and Se SOD activities. No significant difference was observed in liver SOD activities (Fig. 3F). Concerning heart SOD activities (Fig. 3G), no significant difference appeared between C (0.38 ± 0.06 U/mg of tissue), D (0.47 ± 0.10 U/mg of tissue), Spi (0.79 ± 0.15 U/mg of tissue) and SeSP (0.46 ± 0.03 U/mg of tissue). Nevertheless, SOD activities of Se group (1.18 ± 0.12 U/mg of tissue) were significantly higher compared to C, D and SeSP groups but not with Spi group. In soleus (Fig. 3H), SOD activity of C group (0.18 ± 0.06 U/mg of tissue) was not significantly different with Se (0.39 ± 0.05 U/mf of tissue) and SeSP (0.19 ± 0.05 U/mg of tissue). D (0.44 ± 0.05 U/mg of tissue) and Spi (0.41 ± 0.07 U/mg of tissue) groups displayed a higher SOD activity than C and SeSP.

Catalase activities (CAT)

No significant difference appeared in CAT activities between diaphragm (Fig. 4A), long peroneal (Fig. 4B) and gastrocnemius (Fig. 4C). However, in EDL (Fig. 4D), CAT activities of C, D and Se groups were lower than in Spi and SeSP groups. Spi group showed a significant reduction compared to C. CAT activities of C, D and Se groups were not statistically different. Concerning kidney (Fig. 4E), liver (Fig. 4F) there was no significant difference between conditions. In heart (Fig. 4G), SeSP CAT activity was decreased compared to C and D groups. In soleus, no statistical difference appeared between conditions (Fig. 4H).

Selenoprotein expression

In liver, C group (1.04 ± 0.10) displayed a significant higher GPx1 mRNA expression (Fig. 5A) compared to all others conditions. No significant difference appeared between Se (0.58 ± 0.04) and SeSP (0.44 ± 0.07) but GPx1 mRNA levels of these groups were still higher than D (0.10 ± 0.01) and Spi (0.11 ± 0.01) groups. SEPHS2 expression (Fig. 5B), was not modified by our experimental conditions. SelW mRNA expression (Fig. 5C) displayed the same profile than GPx1 with a greater expression in C group (1.02 ± 0.09) compared to the other conditions as well as a higher SelW mRNA expression of the Se (0.68 ± 0.09) and SeSP (0.60 ± 0.04) groups than D (0.22 ± 0.01) and Spi (0.16 ± 0.01) ones. No significant difference appeared between C (1.07 ± 0.14), D (0.72 ± 0.08) and Se (0.75 ± 0.09) groups in SelS mRNA expression (Fig. 5D) even if a tendency to decrease could be observed in D compared to C (p=0.06). D, Se, Spi (0.61 ± 0.07) and SeSP (0.42 ± 0.06) showed no significant difference and C group displayed a SelS mRNA expression increased compared to Spi and SeSP. The same profile was observed in SelP mRNA expression (Fig. 5E) except that no difference appeared between C (1.03 ± 0.09) and SeSP (0.72 ± 0.07). C group (1.08 ± 0.17) TrxR1 mRNA expression (Fig. 5F) was no significantly different from D (0.78 ± 0.05), Se (0.65 ± 0.05) and Spi (0.73 ± 0.05) groups even if Se group displayed a non-significant reduction (p=0.07). SeSP (0.56 ± 0.15) showed a reduced TrxR1 mRNA expression compared to C group while was no different from others.

In Kidney, GPx1 mRNA relative expression (Fig. 6A) of C group was 1.02 ± 0.08. It was identical to Se (1.21 ± 0.08) and SeSP (0.89 ± 0.10) groups even if Se GPx1 mRNA relative expression was significantly increased compared to SeSP. All of these expressions were significantly higher than D (0.52 ± 0.07) and Spi (0.44 ± 0.06). No significant difference was observed between C (1.02 ± 0.08), Se (1.06 ± 0.12), Spi (1.10 ± 0.07) and SeSP (0.80 ± 0.08) SEPHS2 mRNA relative expression (Fig. 6B) while only D (1.45 ± 0.14) displayed an increased expression compared to D and SeSP. SelW mRNA relative expression (Fig. 6C) was not modified by our experimental conditions. No significant variation was shown in C (1.01 ± 0.07), D (1.06 ± 0.09), Spi (1.10 ± 0.06) and SeSP (1.21 ± 0.12) SelT mRNA relative expression (Fig 6D). Only Se group (1.41 ± 0.11) displayed a higher expression compared to C group and a non-significant increase compared to D (p=0.07). In GPx3 mRNA relative expression (Fig. 6E), no significant difference was observed between C (1.07 ± 0.16), Se (0.85 ± 0.11), Spi (0.66 ± 0.03) and SeSP (0.85 ± 0.09) groups. C expression was still higher than D (0.60 ± 0.08) and displayed a non-significant tendency to increase compared to Spi group (p=0.054). No significant difference appeared between any groups in Megalin mRNA relative expression (Fig. 6F). Se (1.93 ± 0.16) TrxR1 mRNA relative expression (Fig. 6G) was significantly higher than all other groups.

In brain, no significant difference was observed in any group for any selenoprotein (Fig. 7A, B, C, D, E and F).

In heart, C (1.03 ± 0.08), D (0.59 ± 0.11) and Se (0.83 ± 0.19) groups displayed no significant difference in GPx1 mRNA relative expression (Fig. 8A) even if a tendency to decrease was observed in D group compared to C (p=0.058). Spi (0.36 ± 0.08) and SeSP (0.44 ± 0.05) groups displayed reduced expression compared to C and Se. Concerning SEPHS2 (Fig. 8B), SelW (Fig. 8C) and SelS (Fig. 8D) mRNA relative expression, no significant difference was observed in any group.

Discussion

Selenium deficiency is found in many pathologies. However, despite numerous studies, selenium physiology is not fully understood. In this study, we have undertaken to compare the selenium repletion and distribution of selenium-enriched spirulina and sodium selenite supplementations after a deficiency. In addition, we analyzed the expression of eleven selenoproteins in different tissues and the activities of the main antioxidant enzymes to understand the impact of these supplementations.

As a preliminary result, we demonstrated that a selenium deficiency was associated with a growth retardation ranging from 17% in the first week, 25% in the second and about 10% in the following weeks. These results are in accordance with the literature [32–34]. Selenium participates in many functions via its incorporation into selenoproteins. Among them, iodothyronine deiodinase are selenoproteins which play a crucial role in thyroid hormones T3 and T4 maturation. These hormones are involved in body weight regulation, development, growth and metabolism [35, 36]. Thus, a selenium deficiency could be responsible for T3 and T4 maturation impairments and lead to growth retardation. Furthermore, Wang and coworkers in 2018 showed that a selenium deficiency could induce a splenic growth retardation by inhibition of the IGF-1 (Insulin Growth Factor 1)/PI3K (phosphatidylinositide 3 kinase)/akt/mTOR pathway which is involved in cell proliferation, survival and metabolism. This pathway is also involved in muscle development. Thus, an inhibition of this molecular pathway could also be responsible for growth retardation. These results have

also been described by Moreno-Reyes and coworkers in 2001 who demonstrated that a selenium-deficiency could lead to bone metabolism impairments and a reduction of circulating GH (Growth Hormone) and IGF-1 [37]. Thus, the mechanisms involved in selenium deficiency-induced growth retardation are multiple and require further studies in order to understand the exact process.

The success of the selenium depletion and the apparition of a Se deficiency is evidenced by a plasmatic selenium concentration at 164.5 ppb for the D group. It has been shown that, such a low $[Se]_{pl}$ corresponds to a selenium deficiency [38]. On this basis, our results showed that Se or SeSP supplementation restore partially or totally selenium concentrations in most of tissues. Indeed, with SeSP supplementation, the selenium concentrations were restored totally in plasma, urine, liver, kidney and soleus and partially in heart. Concerning Se supplementation, the Se concentrations were restored totally in plasma, urine and kidney and partially in liver, heart and soleus. Brain seems to be an exception : indeed, no variation in Se concentration was observed all along the experiment even when rats were fed with Se deficient diet. These results highlighted that the hierarchy of selenium redistribution within the body depends on both the organs and the type of supplementation (Se or SeSP). For example, the heart does not seem to be a priority during supplementation regardless of the selenium form since it is the only organ where selenium concentration is not fully restored following SeSP supplementation. These differences between the organic and inorganic forms could be explained by their distinct absorption and metabolism mechanisms. Indeed, organic selenium forms is mainly found as selenomethionine [39]. It can be absorbed by methionine transporter present on the enterocyte surface [40, 41] in order to join the cellular methionine pool. Then, SeMet will be incorporated by a non-specific mechanism into proteins at the methionine place because the $tRNA^{Met}$ is not able to discriminate Met and SeMet [42]. This phenomenon occurs at a ratio of 1:8000 and could increase up to 1:2800 if the subject is supplemented with SeMet [43]. These proteins are called SeMet-containing proteins. The SeMet incorporation does not interfere with the protein structure, but can greatly affect its activity due to the high reactivity of SeMet compared to Met [42, 44]. The other fate of absorbed SeMet is to be transformed into H_2Se (Selenide) and released into the bloodstream before its metabolization in the liver. Concerning sodium selenite, numerous studies have demonstrated that it is well absorbed but still less than organic selenium [45–47]. Our results confirm the literature and demonstrate that a selenium-enriched spirulina form supplementation is more bioavailable than an inorganic form and allow to restore selenium concentration in most of tissue. Moreover, our results confirm that brain is fully protected from selenium deficiency and emphasize the selenium redistribution hierarchy. Brain has specific receptors named ApoER2 (Apolipoprotein E receptor 2) which are able to bind selenoprotein P, the main carrier of selenium in the bloodstream. The ApoER2 receptors and SelP will be internalized and the selenium will be reused to enable the biosynthesis of new selenoproteins. Thus, the brain is able to maintain a stable selenium concentration by this mechanism called the “SelP cycle” [48, 49].

Regarding antioxidant activities, selenium supplementations completely restored liver and kidney GPx activity. These results are consistent with selenium concentration in both of these tissues. However, sodium selenite did not totally restore selenium concentration while GPx activity was identical to the control group. These results suggest that sodium selenite supplementation was more efficient for improving GPx activity in the liver and emphasized that different selenium forms could be used for different purposes during

supplementation after a selenium deficiency. Moreover, in all the analyzed muscles (either skeletal or cardiac), GPx activity was partially restored after selenium supplementation regardless of the Se form. Therefore, liver and kidney seem to have priority over muscles at least concerning GPx activity which could be explained by the fact that these organs are extremely involved in oxidative stress defense. Interestingly, selenium-enriched spirulina supplementation appeared to be more efficient for improving selenium concentration in tissues but appeared to be less efficient to restore GPx activity. Indeed, in the heart, selenium concentration was identical between Se and SeSP supplementation but GPx activity was better restored in the Se group. Furthermore, in soleus, this pattern was even more pronounced since selenium concentration was similar to the control group while GPx activity was still significantly lower after the supplementation period. These results are partly consistent with those presented by Ringuet and coworkers in 2021. They demonstrated that organic selenium supplementation was more efficient to improve selenium concentration in muscle but not in liver, ileum and plasma at a normal range of selenium [50]. Furthermore, they found a better GPx activity in liver, ileum and plasma with a selenomethionine supplementation compared to inorganic form. However, mice used in this study didn't receive a selenium deficient diet. Concerning SOD activity, our results demonstrated no variation in Long peroneal (PL), gastrocnemius, extensor digitorum longus (EDL), kidney and liver. However, inorganic selenium supplementation was more effective to enhance SOD activity in diaphragm, heart and soleus in order to compensate the GPx activity decreased in these organs. In contrast, CAT activities displayed a different pattern. In fact, no difference between SeSP and inorganic selenium form appeared in diaphragm, PL, gastrocnemius, kidney, liver and soleus while inorganic was better to restore heart CAT activity and organic EDL CAT activity. Our results seemed to highlight that a different pathway occurred after Se and SeSP supplementation. In the heart, both forms of selenium improved total GPx activity without fully restoration. On the other hand, Se seemed to lead to an increase in SOD activity while SeSP induced a reduction in CAT activity. In the soleus, SOD activity is also increased following Se supplementation as in the diaphragm. In contrast, in the EDL, our results indicated an increase in CAT activity following SeSP supplementation. We could hypothesize that these two supplementations may act on different mechanisms to compensate for the GPx activity reduction. Interestingly, it has been shown that similar compensation patterns can be found in fish exposed to a high oxidative stress [51]. Again, tissues and antioxidant enzymes involved are distinct, demonstrating a different fate for administrated selenium forms. Further investigations are required to fully understand mechanisms by which each form acts.

In order to determine the hierarchy operating at the protein expression level, we analyzed the effects of selenium deficiency and then selenium-enriched spirulina or inorganic (sodium selenite) supplementation on selenoprotein expression in liver, kidney, brain and heart. The first mention of this hierarchy was firstly reported by Behne and coworkers in 1988. They showed that, after a selenium deficiency, supplementation of selenium will distribute the element in specific organs in order to allow the expression of some selenoproteins [52]. We already found that some organs were prioritized in our model and were selenium form dependent. Furthermore, we also demonstrated that GPx1 mRNA expression wasn't restored in liver, kidney and heart. These results are consistent with literature which demonstrated that GPx1 is not a priority and could be reduced to give priority to other selenoproteins [21, 43, 53, 54]. However, an inorganic selenium supplementation allowed to restore GPx1 expression in heart contributing to emphasize the specific role of

this form in heart metabolism as described above. Nevertheless, SEPHS2 (selenophosphate synthetase 2) was highly maintained in every tissue. This could be explained by the crucial role of this enzyme in the selenoprotein expression process. In fact, selenium will be transformed into selenide (H_2Se) and then converted by SEPHS2 to monoselenophosphate [55]. It is in this form that it will get incorporated into specific tRNA ($tRNA^{[Ser]Sec}$) before selenoprotein expression. Therefore, preservation of a high level of SEPHS2 is essential to allow selenoprotein expression. Our results demonstrated once again that the brain was fully protected from a selenium deficiency because no modification of expression was observed in any of the analyzed selenoproteins. SelW expression was highly conserved in kidney, brain and heart while in liver, even if both selenium forms supplementation enhanced its expression, it was greatly impacted during selenium deficiency (D group). SelW is ubiquitous but is highly expressed in the brain and muscles [56] where it plays a major antioxidant activity [57]. Furthermore, its antioxidant role is also observed in kidney HEK293 cells [58]. Thus, SelW expression appeared to be important to maintain in brain, kidney and heart while it can be reduced in the liver. SelP was also highly conserved in brain and liver. As mentioned above, SelP is the main blood carrier of selenium. Then, its expression is well preserved to ensure the proper distribution to other organs. Furthermore, SelP is the only selenoprotein to contain multiple selenocysteine residues and is essential to dispatch selenium in the whole body [59]. These results were also confirmed by ApoER2 expression in brain. Interestingly, kidney expressed Megalin, another specific receptor of SelP which is different from ApoER2 [60]. Our results demonstrated that Megalin was not impacted by selenium deficiency and thus that kidney plays an important role in selenium conservation. We initially hypothesized that an increase in megalin expression could be found following selenium deficiency because of the very low amount of selenium found in the urine. Our results showed that this decrease was probably mainly due to the low selenium concentration in the whole organism and not to an adaptation mechanism involving megalin expression. Furthermore, SelS is localized in ER (endoplasmic reticulum) and participates in the ERAD system (Endoplasmic Reticulum-associated protein degradation) involved in targeting and degradation of misfolded protein [61]. Interestingly, selenium deficiency affected its expression and couldn't be restored after a selenium-enriched spirulina supplementation while inorganic supplementation allowed it to restore the same expression found in the control group. However, the heart seemed to be more protected as demonstrated by the absence of variation in a selenium deficiency state. Then, TrxR1 was preserved either in liver and kidney highlighting the high level of hierarchy of this selenoprotein when the organism is selenium deficient. Moreover, inorganic supplementation seemed to increase TrxR1 expression in the kidney compared to the control group.

Conclusion

The physiology of selenium appears to be extremely complex. Our results confirmed the existence of a two-fold hierarchy. On the one hand, in the case of a selenium deficiency and supplementation, selenium will be redistributed to the organs in a different way depending on the supplementation. On the other hand, the expression of selenoproteins constitutes a second level of hierarchy. Moreover, the observed differences between organs evidence the existence of specific regulation mechanisms. Further studies are needed to understand the ins and outs of this phenomenon. Finally, this study shows that different selenium forms could be used for different purposes during supplementation since inorganic selenium (sodium selenite) will

have a greater impact on antioxidant enzyme activities and selenoprotein expression, whereas supplementation with a selenium-enriched spirulina will allow a better restoration of selenium concentrations in tissues.

Declarations

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Castel T[1], Théron M[1], Pichavant-Rafini K[1] and Léon K[1] conceived and designed the experiments and contributed to the writing and revising of the article manuscript. Gandubert C[1], Amérand A[1], Guernec A[1] and Gueguen B[2,3] contributed to the acquisition, the analyse of the data and the revision of manuscript. All authors have seen and approved the final manuscript.

Ethical approval

Our animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. National specific laws have been observed too.

Consent to participate

Not applicable

Consent to publication

Not applicable

Availability of data and material

The authors declare that data and material are available

Code availability

The authors declare that software application and custom code are available

Compliance with Ethical Standards

Research involving animals

Our animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. National specific laws have been observed too.

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Figures

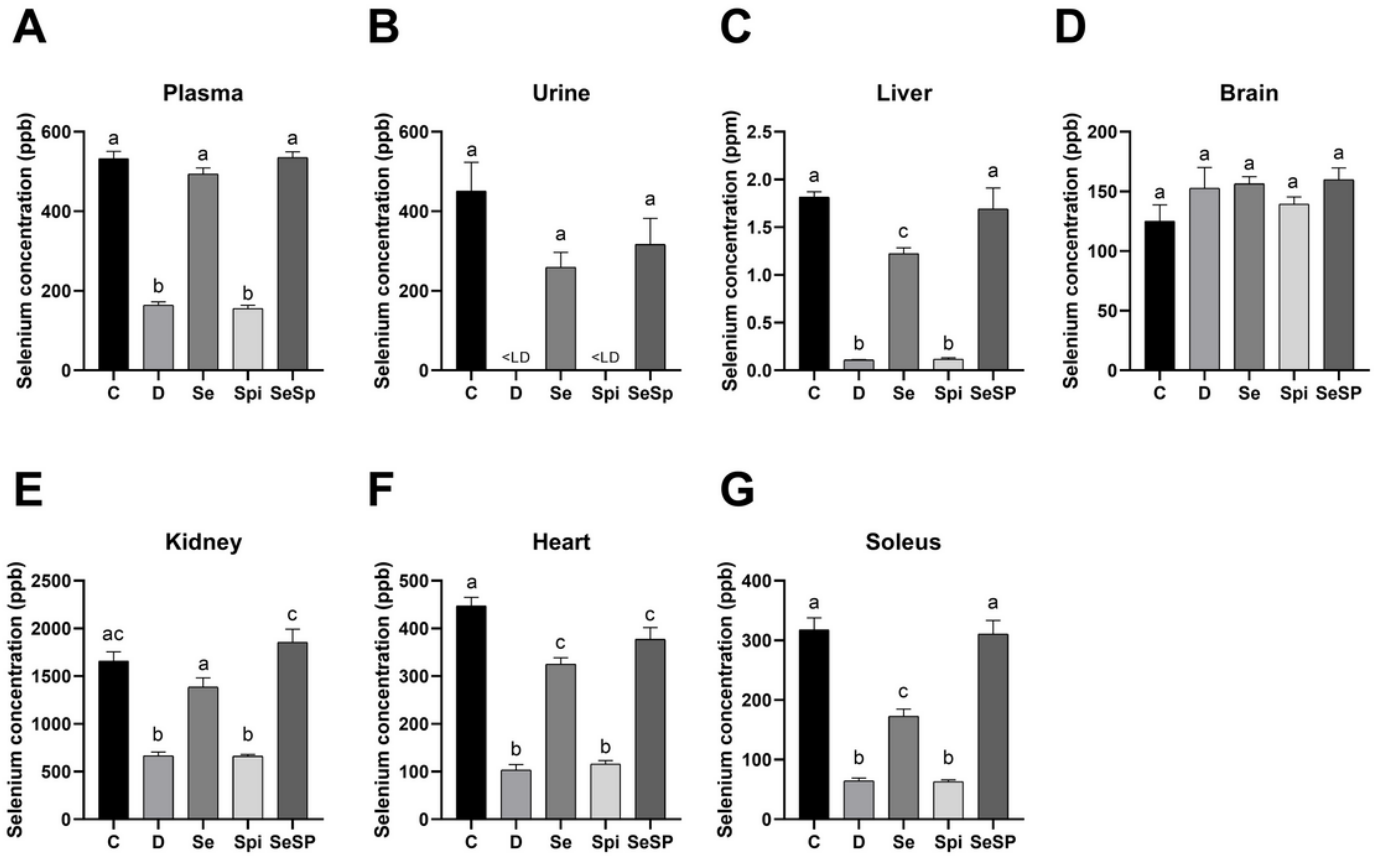

Figure 1

Selenium concentrations in tissues. Plasmatic (A), Urinary (B), liver (C), brain (D), kidney (E), heart (F) and soleus (G). Selenium concentration was measured by ICP-MS in all different groups after 12 weeks of experiment. C, Control Group; D, deficient group; Se, selenium supplemented group; Spi, Spirulina supplemented group; SeSP, selenium-enriched Spirulina supplemented group. Results are presented as mean ± SEM (n=8 for each group). Different letters indicate a significant difference (p-value <0.05).

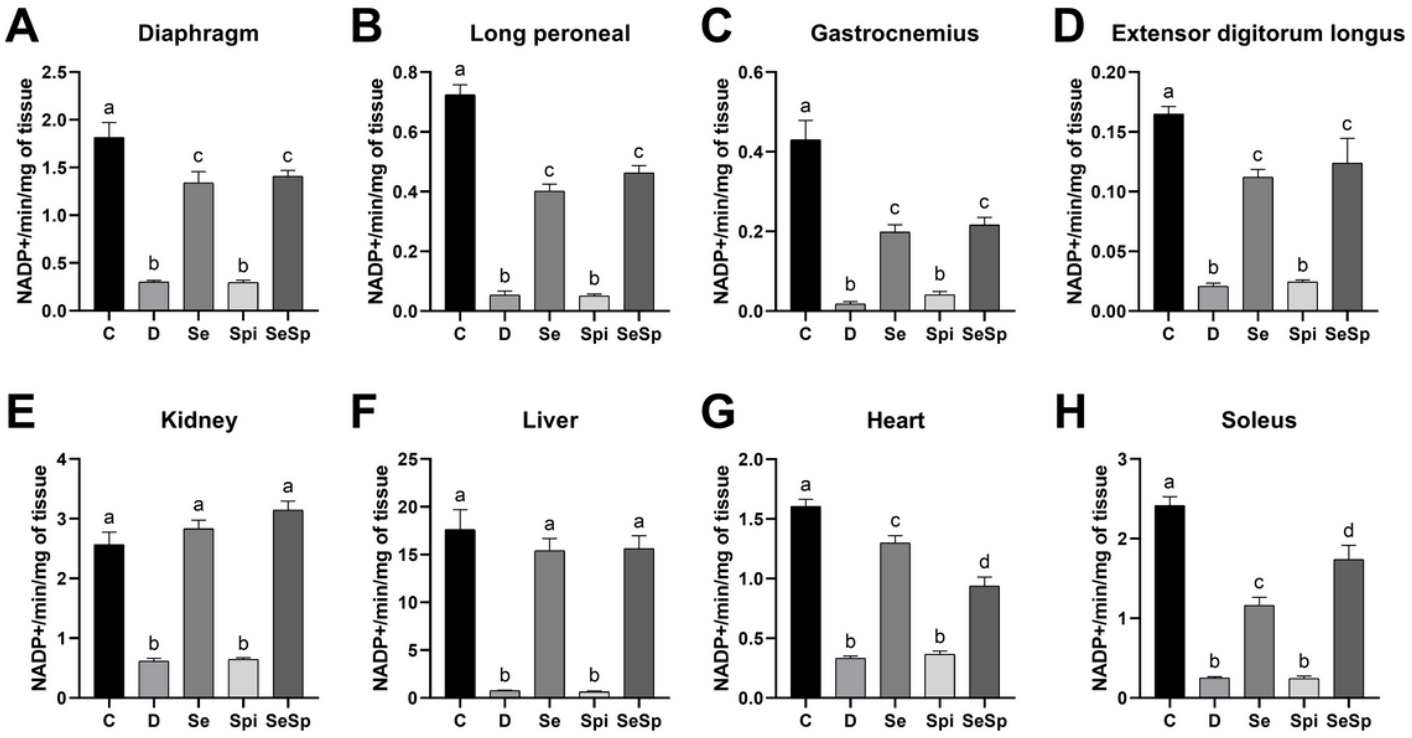

Figure 2

GPx activities in diaphragm (A), long peroneal (B), Gastrocnemius (C), EDL (D), kidney (E), liver (F), heart (G) and soleus (H) after 12 weeks of experiment. C, Control Group; D, deficient group; Se, selenium supplemented group; Spi, Spirulina supplemented group; SeSP, selenium-enriched Spirulina supplemented group. Results are presented as mean ± SEM (n=8 for each group). Different letters indicate a significant difference (p-value <0.05).

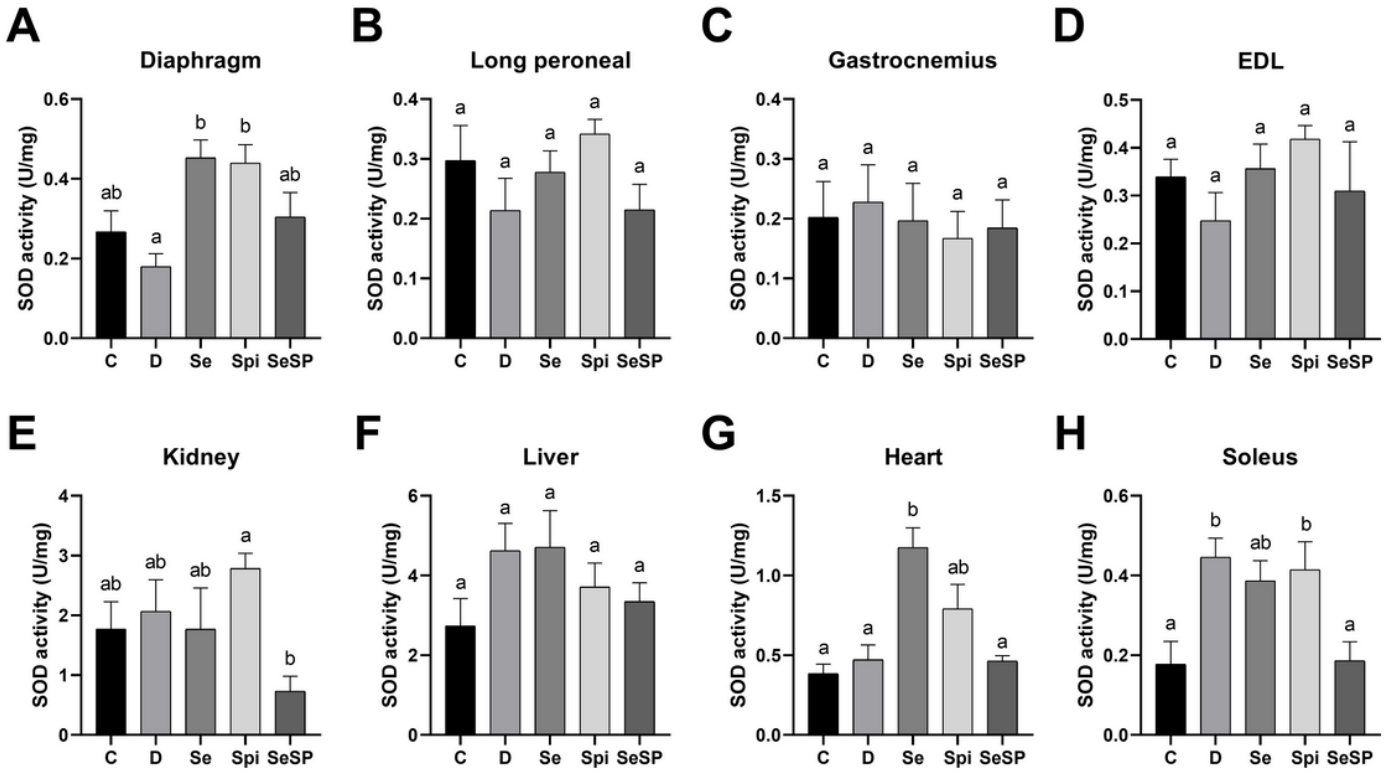

Figure 3

SOD activities in diaphragm (A), Long peroneal (B), Gastrocnemius (C), EDL (D), kidney (E), liver (F), heart (G) and soleus (H) after 12 weeks of experiment. C, Control Group; D, deficient group; Se, selenium supplemented group; Spi, Spirulina supplemented group; SeSP, selenium-enriched Spirulina supplemented group. Results are presented as mean ± SEM (n=8 for each group). Different letters indicate a significant difference (p-value <0.05).

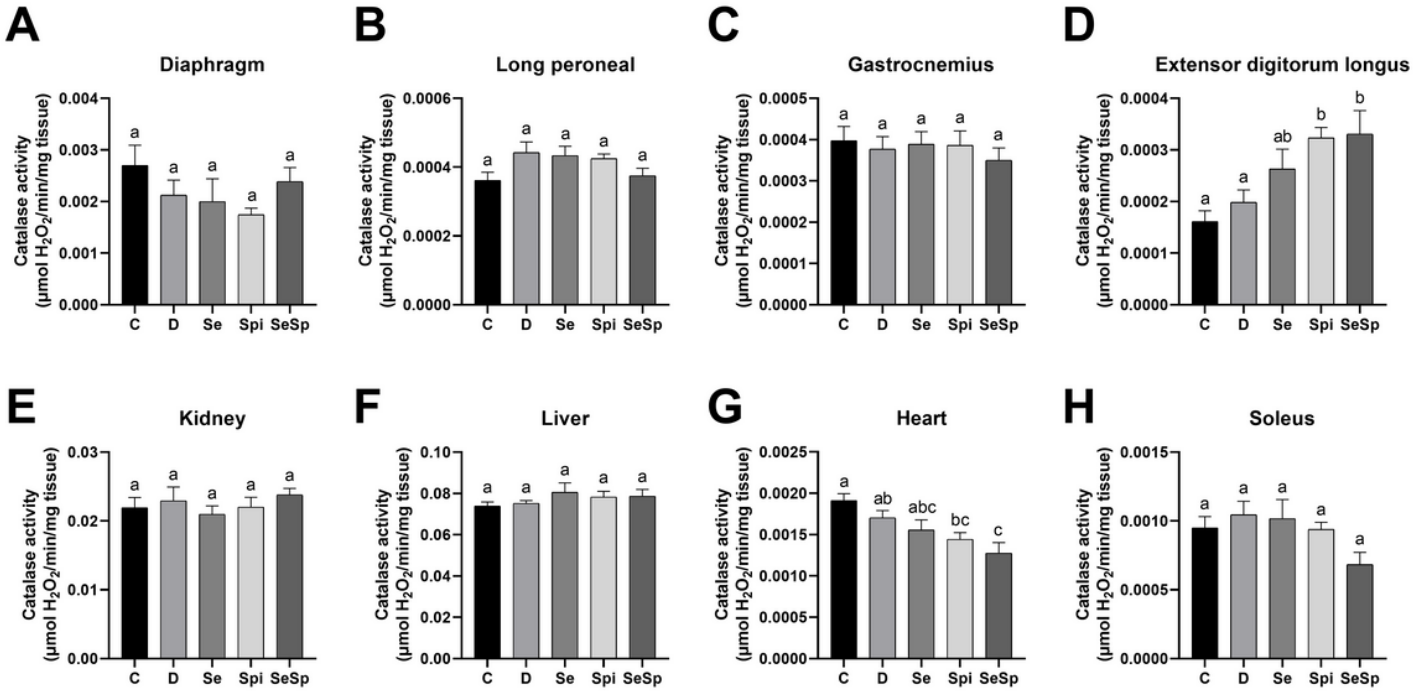

Figure 4

CAT activities in diaphragm (A), Long peroneal (B), Gastrocnemius (C), EDL (D), kidney (E), liver (F), heart (G) and soleus (H) after 12 weeks of experiment. C, Control Group; D, deficient group; Se, selenium-supplemented group; Spi, Spirulina supplemented group; SeSP, selenium-enriched Spirulina supplemented group. Results are presented as mean ± SEM (n=8 for each group). Different letters indicate a significant difference (p-value <0.05).

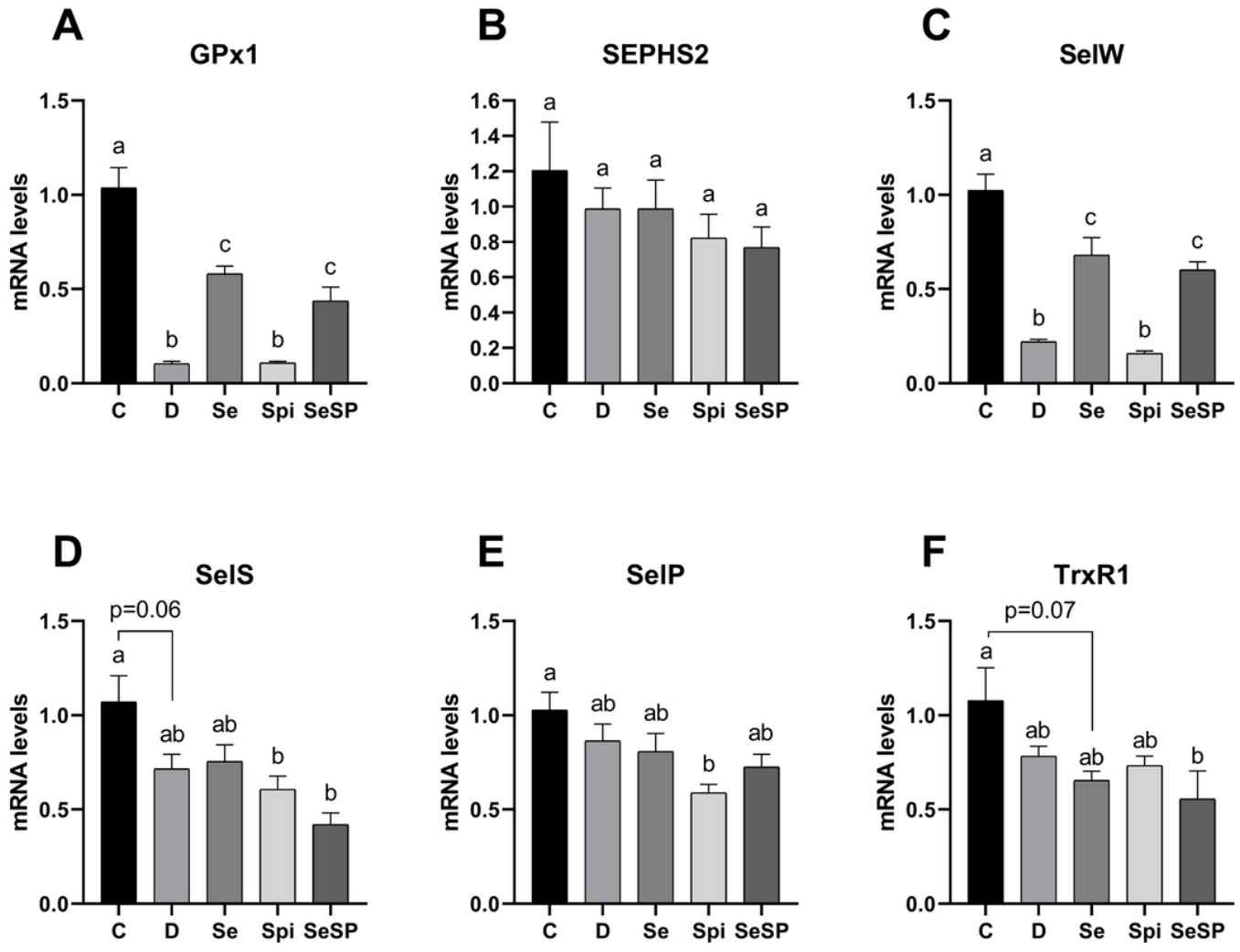

Figure 5

Selenoprotein expression in liver. GPx1 (A), SEPHS2 (B), SelW (C), SelS (D), SelP (E) and TrxR1 (F) relative mRNA levels were quantified in liver after 12 weeks of experiment. C, Control Group; D, deficient group; Se, selenium-supplemented group; Spi, Spirulina supplemented group; SeSP, selenium-enriched Spirulina supplemented group. Results are presented as mean ± SEM (n=8 for each group). Different letters indicate a significant difference (p-value <0.05).

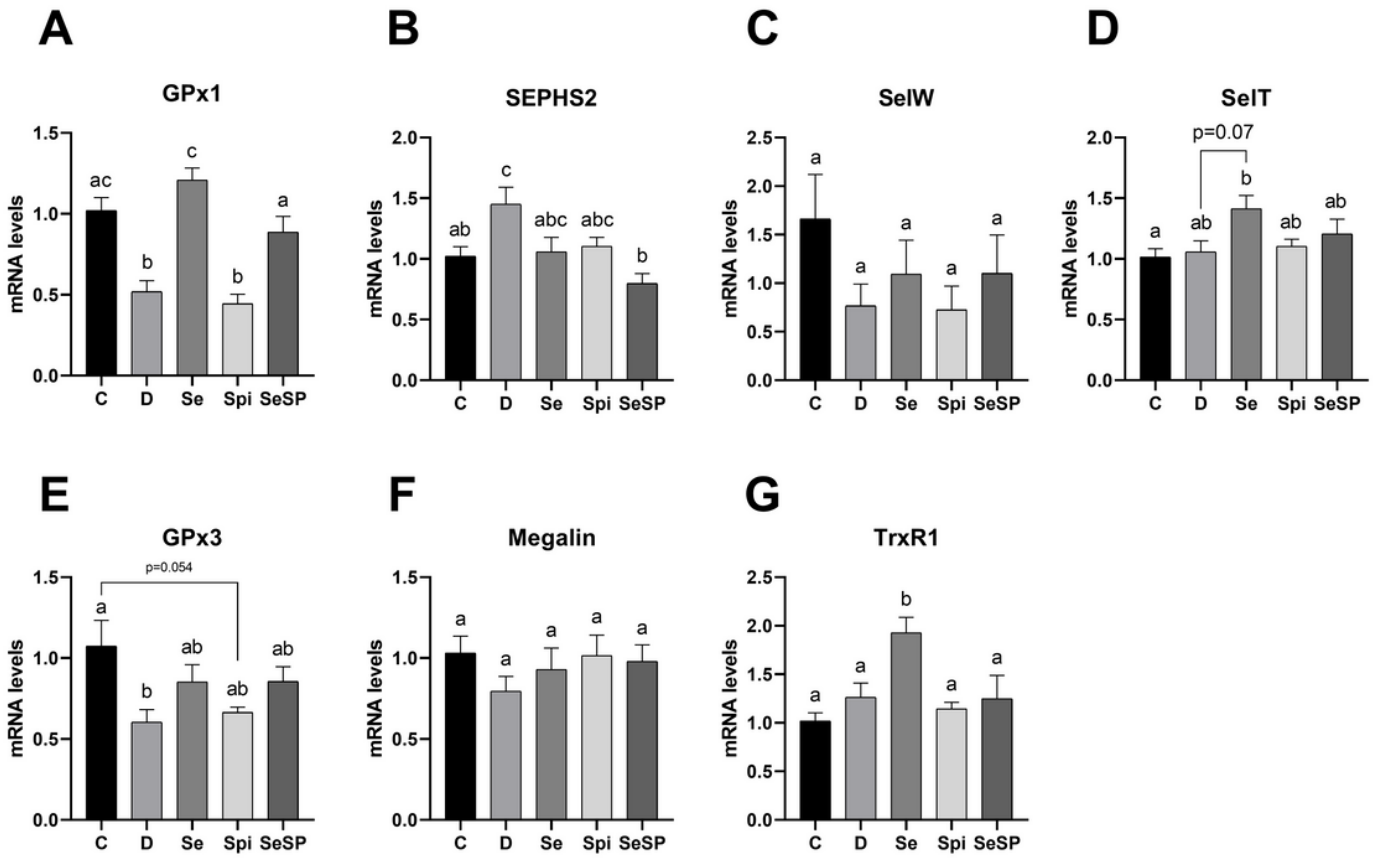

Figure 6

Selenoprotein expression in kidney. GPx1 (A), SEPHS2 (B), SelW (C), SelT (D), GPx3 (E), Megalin (F) and TrxR1 (G) relative mRNA levels were quantified in kidney after 12 weeks of experiment. C, Control Group; D, deficient group; Se, selenium-supplemented group; Spi, Spirulina supplemented group; SeSP, selenium-enriched Spirulina supplemented group. Results are presented as mean ± SEM (n=8 for each group). Different letters indicate a significant difference (p-value <0.05).

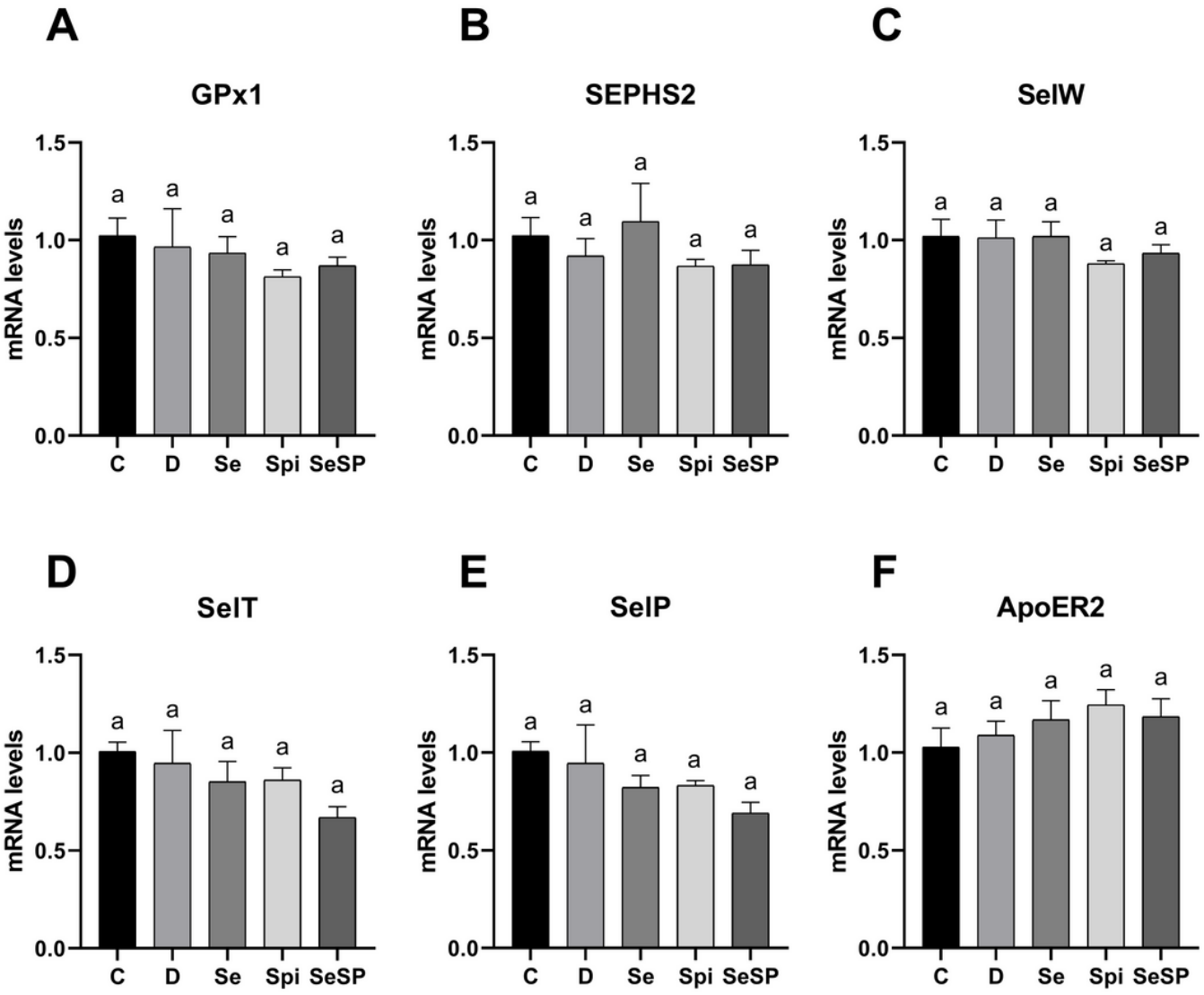

Figure 7

Selenoprotein expression in brain. GPx1 (A), SEPHS2 (B), SelW (C), SelT (D), SelP (E) and TrxR1 (F) relative mRNA levels were quantified in brain after 12 weeks of experiment. C, Control Group; D, deficient group; Se, selenium-supplemented group; Spi, Spirulina supplemented group; SeSP, selenium-enriched Spirulina supplemented group. Results are presented as mean ± SEM (n=8 for each group). Different letters indicate a significant difference (p-value <0.05).

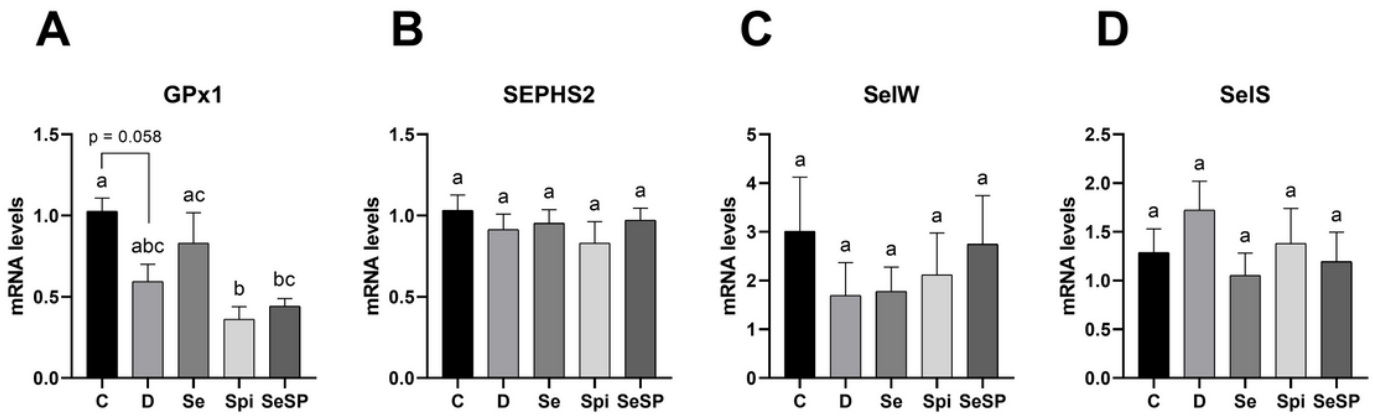

Figure 8

Selenoprotein expression in heart. GPx1 (A), SEPHS2 (B), SelW (C) and SelS (D) relative mRNA levels were quantified in heart after 12 weeks of experiment. C, Control Group; D, deficient group; Se, selenium-supplemented group; Spi, Spirulina supplemented group; SeSP, selenium-enriched Spirulina supplemented group. Results are presented as mean ± SEM (n=8 for each group). Different letters indicate a significant difference (p-value <0.05).