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Low-Se Diet Can Affect Sperm Quality and Testicular Glutathione Peroxidase-4 activity in Rats

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Abstract

This study aimed to observe the influence of selenium (Se) deficiency on sperm quality and selenoprotein expression in rats. Four-week male Wista rats were randomly divided into three groups: Se-A, Se-L, and Se-D (respectively for Se- adequate, low, and deficient group). After 9 weeks, the rats were sacrificed by anesthesia, with the cauda epididymidis quickly fetched for sperm count, motility, and deformity. Meanwhile the blood, liver, brain, heart, and testis were collected for Se and biochemical analysis. It was found that the rats in Se-D had poor growth, while the Se concentrations in blood, liver, and heart for Se-D decreased significantly, compared with Se-A and Se-L ($p < 0.01$). But no significant difference was observed in testis and brain and also no statistical significance for sperm count. The sperm motility for Se-A (63.07%) was significantly higher than Se-L (53.91%) and Se-D (54.15%). Deformities were observed in both Se-L and Se-D. Both glutathione peroxidases (GPxs) and selenoprotein-P (SEPP1) levels in plasma and tissues of Se-D were significantly lower than those of Se-A and Se-L ($p < 0.01$). The SEPP1 levels in heart and brain of Se-L were lower than Se-A ($p < 0.01$). There was no statistical difference for GPx1 between Se-A and Se-L. The GPx4 level in testis of Se-L was lower than Se-A ($p < 0.05$). However, the SEPP1 in plasma, liver, testis, and the GPx3 level in plasma of Se-L were higher than those of Se-A ($p < 0.05$ or $p < 0.01$). Our results show that dietary Se deficiency could reduce GPx4 and SEPP1 expression in testis, which further influence sperm motility and may cause sperm deformity. Selenoprotein expression in some tissues of Se-L was higher than that of Se-A, but sperm quality and GPx4 expression in testis were not improved for Se-L. Low active pseudoselenoproteins might be synthesized in low-Se condition. The underlying mechanism needs to be further investigated.

Keywords Selenium · Glutathione Peroxidase 4 · Selenoprotein-P · Sperm quality

Selenium (Se) is an essential micronutrient for mammals to maintain overall health [1]. Se deficiency has been recognized as a contributing factor to pathophysiological conditions, such as heart disease [2], neuromuscular disorders [3], reproduction [4], and numerous other disorders. Previous studies demonstrated the essential role of Se in spermatogenesis and male fertility in mammals [5]. Se exerts its nutritional functions in the form of amino acid Sec inserted into a group of selenoproteins. Several review literatures reported a strong implication of Se and selenoproteins in mammalian reproduction [6, 7]. The well-characterized selenoproteins with essential functions are selenoprotein-P (SEPP1) and glutathione peroxidases (GPxs), while the latter includes GPx1 and GPx4.

GPx4 is an intracellular selenoprotein that directly reduces peroxidized phospholipids production in cell membranes, also known as phospholipid hydroperoxide GPx [8]. It is distinctly expressed in testes, comprised of cytosolic protein, mitochondrial protein (mGPx4) and nuclear protein (nGPx4), and constituting over 50% of mitochondrial capsule in midpiece of mature sperm [9]. In the early stage of spermatogenesis, GPx4 is believed to protect the developing sperm from oxidative stress-induced DNA damage. In the later phase, through cross-linkage with proteins in midpiece region, however, it provides the integrity to the sperm midpiece by becoming a

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structural component of mitochondrial sheath circumventing the flagellum, which is an essential component for sperm stability and motility [10]. Similarly, SEPP1 plays an essential role in male reproductive functions. It serves as a transport protein of Se, expressed in vesicle like structures in the basal region of the Sertoli cells [11]. Besides, SEPP1 mRNA was also expressed in Leydig cells of rats. The largest loss of Se was manifested in SEPP1 KO models, where a 77% decrease of Se was observed [12].

The foregoing evidences highlight that Se performs significant functions in the male reproductive system regulated by selenoproteins, especially GPX4 and SEPP1. Therefore, it is advisable to perform more studies focusing on the elucidation of additional roles played by GPX4 and SEPP1 in male reproductive functions. In the present study, we observed the influences of different dietary Se (adequate, low, and deficient) on the sperm quality, Se content of blood and tissues, and selenoproteins level, to investigate the important role of Se in male fertility via GPx4 and SEPP1.

Materials and methods

Experimental groups and diet regimes

Thirty-six of four-week SPF male Wista rats (Beijing HFK Bioscience Co., LTD, China) were feed with a standard housing environment. Following 1-week adaptation period, the rats were randomly divided (n = 12 each) into three groups: Se-adequate (Se-A), Se-low (Se-L), and Se-deficient (Se-D). According to the literatures [27, 28], Se supplements for Se-D and Se-A groups were 0.01 (no sodium selenite added) and 0.3 mg Se/kg, respectively. We took the middle value 0.15 mg Se/kg as Se-L. The diet fed to the animals in this study was supplied by Beijing HFK Bioscience Co., LTD, China. The license key of animal fodder was SCXK(Beijing)2014-0008. The rats of Se-A group were fed with the basal feed (1022). The rats of Se-L and Se-D groups were fed with custom-made feed (M1003G). Its composition was given in Table 1 and Table 2. The actual measured Se levels in this study were 0.37, 0.13, and 0.01 mg Se/kg for Se-A, Se-L, and Se-D groups, respectively. The weights were recorded once a week. After feed for 9 weeks, the rats were sacrificed by anesthesia, and the cauda epididymidis were quickly fetched for sperm count, motility, and deformity detections. Blood was taken from the abdominal aorta. The liver, testis, heart, and brain tissues were collected for Se, SEPP1, and GPx4 analysis. All procedures used in this study were approved by the Animal Care and Use Committee of National Institute of Nutrition and Health, Chinese Centre for Disease Prevention and Control.

Table 1 The composition of Salt Mixture (M1003G)

Ingredient	M1003G (Se-D) g/kg	M1003G (Se-L)
$CaCO_3$	357	357
KH_2PO_4	196	196
$K_3C_6H_5O_7 \cdot H_2O$	70.78	70.78
K_2SO_4	46.6	46.6
MgO	24	24
NaCl	74	74
$CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$	0.33	0.33
KIO_3	0.0078	0.0078
$FeC_6H_5O_7 \cdot 5H_2O$	6.39	6.39
$MnCO_3$	0.68	0.68
Na_2SeO_4	0	0.0064
$ZnCO_3$	1.53	1.53
$CrK(SO_4)2 \cdot 12H_2O$	0.275	0.275
$(NH_4)Mo_7O_{24} \cdot 4H_2O$	0.008	0.008
$Na_2SiO_3 \cdot 9H_2O$	1.64	1.64
$LiCl \cdot H_2O$	0.0257	0.0257
H_3BO_3	0.0815	0.0815
NaF	0.0635	0.0635
$NiCO_3 \cdot 2Ni(OH)_2 \cdot 4H_20$	0.0357	0.0357
NH_4VO_3	0.0066	0.0066
Sucrose	220.546	220.546
Total	1000	1000

Detection of sperm count, motility and deformity

Sperm count and motility: The method was based on an epididymal sperm count protocol [13]. The sperm solution

Table 2 The composition of the basal diet (M1003G)

Ingredient	M1003G (Se-D) g/Kg	M1003G (Se-L)
Casein	200	200
Cystine	3	3
Starch	397	397
Maltodextrin	132	132
Sucrose	100	100
Cellulose	50	50
Bean oil	70	70
Tertiary butylhydroquinone (TBHQ)	0.014	0.014
M1003G (Se-D)	35	
M1003G (Se-L)		35
Vitamin complex (V1002)	10	10
Choline bitartarate	2.5	2.5
Total	1000	1000

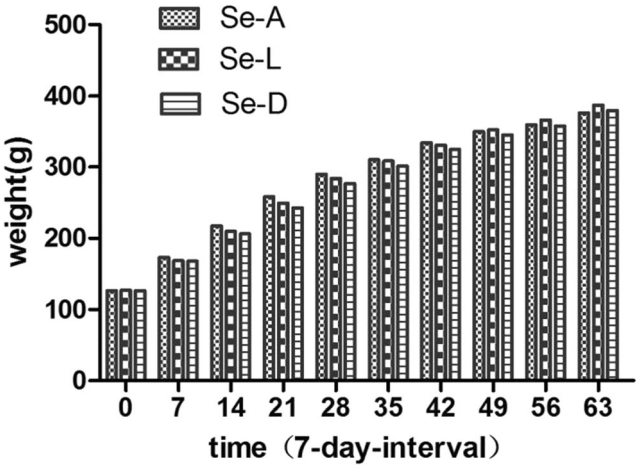

Fig. 1 Weight gain graph of rats. Se-A: Se-adequate. Se-L: Se-low. Se-D: Se-deficient. $n = 12$

was immediately prepared after sample collection (n = 10–12), and the cells were loaded into a 100-mm-deep hemocytometer chamber (Thermo Fisher). The videos of the 4 corner squares of the chamber were recorded using a microscope (Nikon TS100) at 4003 magnification for 10 s. We then evaluated the sperm to assess their progressive motility (PM), no-progressive motility (NPM), and total motility (PM + NPM) with reference to the methods recommended by the WHO.

Deformity analysis: The methods for pathologic slide preparation and morphology analysis were obtained from the study by Seed et al [14]. Duplicate slides were prepared for each sperm sample (n = 6/group). We then randomly selected 200 sperm cells from each slide, and the cells were counted at 4003 amplification. The deformity rate (percentage) was calculated as (number of deformed sperm/total number of sperm) x100%.

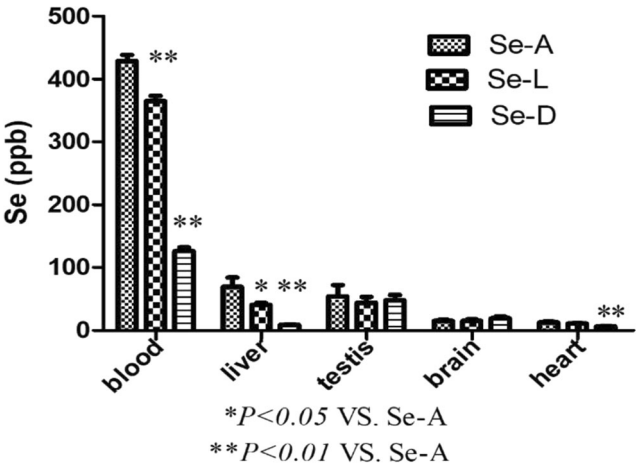

Fig. 2 Se concentrations in blood, liver, testis, brain and heart of rats. Se-A: Se-adequate. Se-L: Se-low. Se-D: Se-deficient. n = 8. *$p < 0.05$, **$p < 0.01$, Data represent the mean ± standard error ($\bar{x}$ ±s)

Se detection of whole blood and tissues in rats

For the analysis of whole blood and tissue Se, 1 g of a previously heated (25 °C) and shaken blood and tissue samples were weighed into 10-mL Teflon microwave vessels and 2 mL of 65% HNO_3 was added. During digestion, the samples were digested at 120 °C for 10 min, after which the temperature was ramped to 120 °C (within 8 min), and then to 160 °C for 10 min, and finally to 175 °C for 20 min using a CEM MARS Xpress microwave system (CEM, Matthew, NC, USA). The cooled, digested samples were diluted to 10 mL with ultrapure water and analyzed for total Se content by inductively coupled plasma mass spectrometry (ICP-MS).

Double-antibody sandwich ELISA assay for SEPP1 and GPx

After cutting samples, check the weight and add cold PBS (PH7.4), and maintain samples at 2–8 °C after melting. The samples were homogenized by hand or grinders and centrifugated for 20 min at the speed of 2000–3000 rpm. The supernatant was collected for detection. The double-antibody sandwich ELISA assay for quantification of SEPP1 and GPx was used according to the instructions of a validated SEPP1/GPx-specific ELISA kit [15, 16]. Setting five standards for drawing calibration curve, the absorbance was measured using a microplate reader at a wavelength of 450 nm.

Statistical analysis

Data were analyzed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). All values are expressed as Mean ± SEM. Comparisons between groups were statistically evaluated by Student's test or one-way ANOVA with a post hoc Fisher's test. A probability of $p < 0.05$ was considered to be statistically significant.

Results

Weight change of Rats

Their average weights were weekly given in Fig. 1. For the forgoing 6 weeks, the weight gains of the Se-A, Se-L, and Se-D groups were 207.88, 204.05, and 198.24 g, respectively. During the next 3 weeks, the weight gains became 41.74, 56.41, and 55.25 g, respectively. Compared with the Se-A and Se-L groups, the weight gain of rats in Se-D group was slow for the forgoing 6 weeks, but fast for the next 3 weeks.

Table 3 Density, motility, and deformity of sperm ($n = 12$, $\bar{x} \pm s$)

Group	Sperm density(10^6/ml)	Sperm activity(%)
Se-A	15.5 ± 3.88	63.07 ± 10.97
Se-L	15.32 ± 3.53	53.91 ± 7.34*
Se-D	16.54 ± 4.58	54.15 ± 8.51*

Compared with Se-A and Se-L groups * $p < 0.05$

Se concentrations in blood, liver, testis, brain, and heart of rats

Compared with Se-A group, Se concentrations in blood and liver were significantly decreased for Se-L and Se-D groups ($p < 0.05$ or $p < 0.01$), as compared with Se-L group for Se-D group ($p < 0.01$). Se concentration in heart for Se-D group was lower than Se-L group ($p < 0.01$) (Fig. 2). Se concentrations in blood, liver, and heart were significantly decreased in Se-D group. There were no significant differences for Se concentrations in testis and brain.

Sperm density, motility, and deformity

As shown in Table 3, there was no statistic difference for sperm density. The sperm motility in Se-A is 63.07%, significantly higher than that in Se-L (53.91%) and Se-D (54.15%) groups. There were observed deformities in Se-L and Se-D groups, but no deformity in Se-A group was found (Fig. 3). Both sperm motility and morphology were affected for Se-L and Se-D groups.

Double-antibody sandwich ELISA assay for SEPP1 and GPxs level in plasma and tissues

As shown in Fig. 4, the SEPP1 levels in plasma and tissues of Se-D group were significantly lower than those in Se-A and Se-L groups ($p < 0.01$). The SEPP1 levels in heart and brain of Se-L group were lower than Se-A group ($p < 0.01$). However, the SEPP1 levels in plasma, liver, and testis of Se-L group were higher than those of Se-A ($p < 0.05$ or $p < 0.01$).

The GPx levels in plasma, liver, and testis of Se-D group were significantly lower than Se-A and Se-L groups ($p < 0.01$). There was no difference for GPx1 in liver between Se-L and Se-A group. The GPx4 level in testis of Se-L was lower than Se-A ($p < 0.05$). However, the GPx3 level in plasma of Se-L was higher than that of Se-A ($p < 0.01$).

The GPx3 level in plasma and the SEPP1 levels in plasma, liver, and testis for Se-L group were significantly higher than those of Se-A group, but the GPx4 level in testis of Se-L was lower than that of Se-A.

Discussion

Selenium (Se) is an important micronutrient for animal and human health. More and more relevant evidences suggest that Se is essentially required for spermatogenesis and male fertility, presumably because of its vital role in modulation of antioxidant defense mechanisms and other essential biological pathways [17, 18]. It was shown that the alterations in Se levels might perturb the redox status and could lead to oxidative stress, adversely affecting male fertility by altering the expression of biologically important markers and activity of antioxidant enzymes [19, 20].

Se concentration and GPx4 activity (in testis) were significantly reduced when mice fed with Se-deficient dietary (0.02 ppm) for 4 months [21]. Se deficiency resulted in a significant decrease in body and testicle weights of the chicks [22]. Our result showed that weight gain was slow for Se-deficient rats. Compared with Se-adequate and Se-low groups, Se concentrations in blood, liver, and heart were significantly decreased in Se-deficient group, but not in testis and brain, probably because of the prior Se utilization for different tissues in Se-deficiency condition [23]. Accordingly, both sperm motility and morphology were affected when the rats were fed with Se-deficient fodder for 8 weeks. These results were in line with previous reports that sperm from Se-deficient (0.02 ppm) mice demonstrated vitiated chromatin condensation and declined in vitro fertilization ability compared to the Se-sufficient (0.2 ppm) mice [24].

As an essential component of selenoproteins, Se plays both structural and enzymic roles, being well known for its

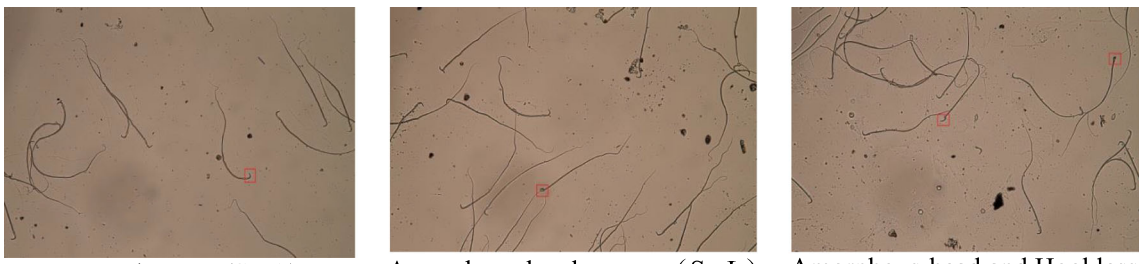

Fig. 3 Sperm morphology of rats. Se-A: Se-adequate. Se-L: Se-low. Se-D: Se-deficient. n = 8.The red boxes in the figure show the normal and abnormal sperm morpholpgy

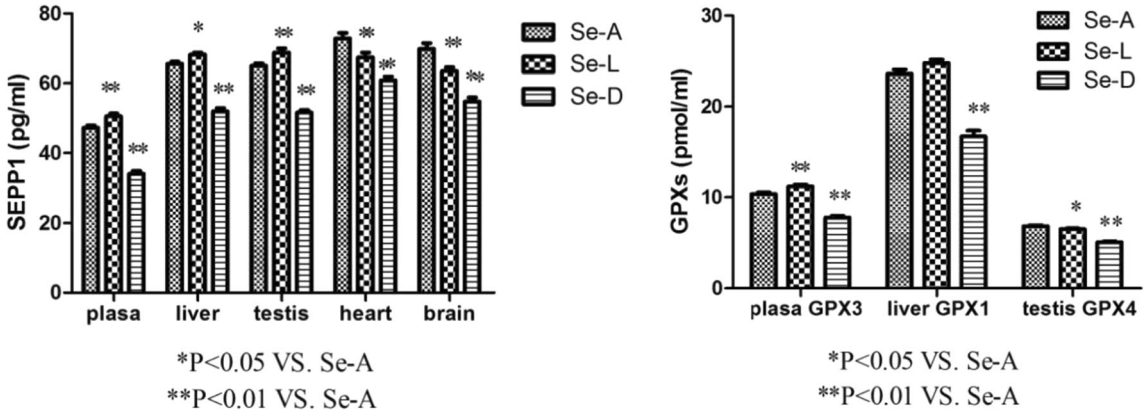

Fig. 4 SEPP1 and GPxs concentrations in plasma and tissues. Se-A: Se-adequate. Se-L: Se-low. Se-D: Se-deficient. $n = 8$. *$p < 0.05$, **$p < 0.01$, Data represent the mean ± standard error ($\bar{x}$ ±s)

catalytic and antioxidative functions. GPx4 is the major selenoprotein expressed by germ cells in the testis, having multiple functions and representing the pivotal link between selenium, sperm quality, and male fertility. The deletion of mGPx4 causes male infertility, conferring the vital role of selenium in mammalian male fertility [25]. Homozygous expression of GPx4 with a targeted substitution of selenocysteine to serine causes early embryonic death but male subfertility in heterozygous mice [26]. Zhou et al. reported Se deficiency showed a lower expression of sensitive antioxidant selenoproteins (GPx1 and Txnrds), but excessive doses of Se-impaired sperm quality and this were linked with reduced mRNA expression of nGPx4 [27]. Our result showed that liver GPx1, testis GPx4, and plasma GPx3 activities were all significantly decreased for Se-D groups compared with Se-A and Se-L group, as reported in literatures that dietary selenium deficiency resulted the mRNA levels of some selenoprotein genes and the activities of GPxs decreased both in the spleen of pigs [28] and chicken aorta vessels [29].

Recently, another fertility-related marker, the selenoprotein P (SEPP1), was highlighted [30]. SEPP1 is a plasma protein, required for selenium supply to the testis. Liver-derived SEPP1 can bind apolipoprotein E receptor 2 on epithelial cell (Sertoli cells) membranes in testes to deliver selenium to organs [31], while testicular SEPP1 is locally and exclusively expressed in Leydig cells [32]. Our result showed that, in plasma and tissues, SEPP1 concentrations of Se-D group were significantly less than that of Se-A and Se-L groups, supplying more evidence for SEPP1 as a fertility-related marker.

It was reported that up to 10% selenocysteine (Sec) sites were occupied by cysteine (Cys) in selenoprotein (Thioredoxin Reductase 1, TR1) with a lower activity in mice fed with normal amounts of dietary selenium [33]. And then the same research group found that Cys might be inserted

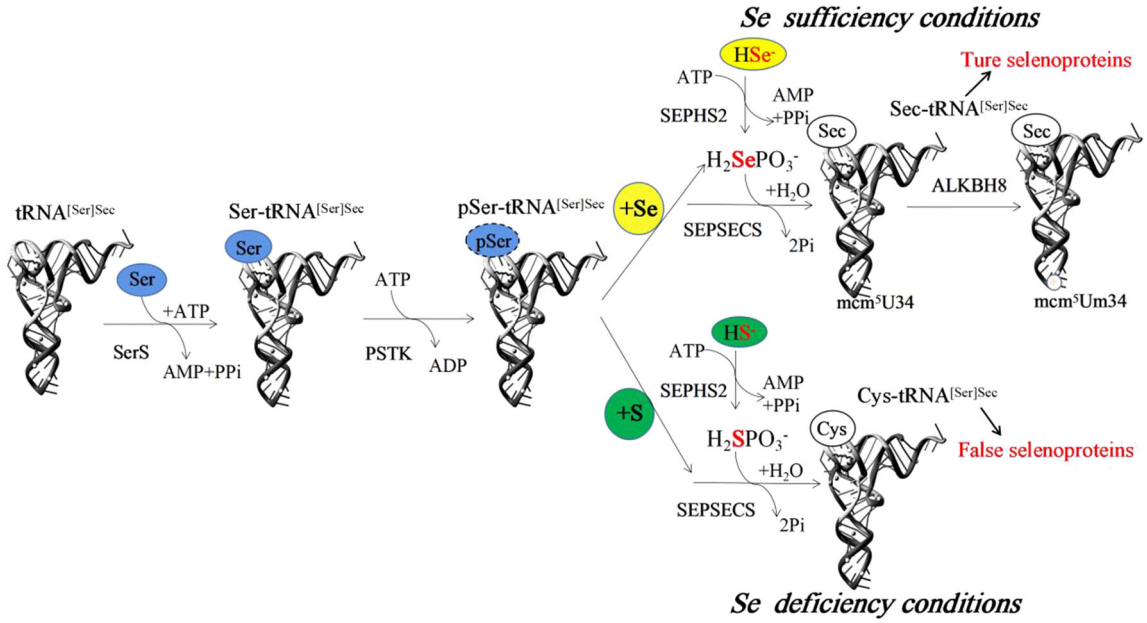

Fig. 5 This schematic illustrates the de novo synthesis of SeCys and Cys with the presence of codon UGA. Modified from Reference [36]

in place of various Sec residues in SEPP1 in human plasma [34]. And more, someone reported that Sec/Cys (U46C) mutant rescued cell death of *GPx4*$^{-/-}$ cells, whereas the Sec/Ser (U46S) mutant failed [35]. In this study, we have noticed that SEPP1 expressions of Se-L group in several tissues (plasma, liver and testis) were significantly higher than that of Se-A together without an optimal activity of GPx4 and the normal form of sperm. As shown in Fig. 5, the *de novo* biosynthesis of Cys was found to be decoded by the same codon UGA as that of SeCys [36]. Hence, we speculated there was a physiological adaptation to low-Se by replacing SeCys with Cys to synthesize low active pseudoselenoproteins and maintain the basic physiological functions. That is to say, physiological adaptation to low-Se might not safeguard the normal function of sperm. In the next study, we will further verify the replacement between Sec and Cys residue in SEPP1 from mice feed with different amounts of dietary selenium and human tissue from people in different Se-level areas.

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The authors' responsibilities were as follows QW and SZ completed the experiments and wrote the paper; YQL and FH contributed to the detection of Se; LLS and CH participated in the animal experimental; WPM and JZC provided valuable advice on the writing; ZWH designed the experiments and revised the paper critically for important content; and all authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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