

Selenoprotein Gene Expression in Thyroid and Pituitary of Young Pigs Is Not Affected by Dietary Selenium Deficiency or Excess^{1–3}

Ji-Chang Zhou,⁴ Hua Zhao,⁴ Jun-Gang Li,⁴ Xin-Jie Xia,^{4*} Kang-Ning Wang,⁴ Ya-Jun Zhang,⁴ Yan Liu,⁴ Ying Zhao,⁴ and Xin Gen Lei^{4,5*}

⁴International Center of Future Agriculture for Human Health, Sichuan Agricultural University, Chengdu, Sichuan 611134, China and ⁵Department of Animal Science, Cornell University, Ithaca, NY 14853

Abstract

Expression and function of selenoproteins in endocrine tissues remain unclear, largely due to limited sample availability. Pigs have a greater metabolic similarity and tissue size than rodents as a model of humans for that purpose. We conducted 2 experiments: 1) we cloned 5 novel porcine selenoprotein genes; and 2) we compared the effects of dietary selenium (Se) on mRNA levels of 12 selenoproteins, activities of 4 antioxidant enzymes, and Se concentrations in testis, thyroid, and pituitary with those in liver of pigs. In Experiment 1, porcine *Gpx2*, *Sephs2*, *Sep15*, *Sepp1*, and *Sepp1* were cloned and demonstrated 84–94% of coding sequence homology to human genes. In Experiment 2, weaning male pigs ($n = 30$) were fed a Se-deficient (0.02 mg Se/kg) diet added with 0, 0.3, or 3.0 mg Se/kg as Se-enriched yeast for 8 wk. Although dietary Se resulted in dose-dependent increases ($P < 0.05$) in Se concentrations and GPX activities in all 4 tissues, it did not affect the mRNA levels of any selenoprotein gene in thyroid or pituitary. Testis mRNA levels of *Txnrd1* and *Sep15* were decreased ($P < 0.05$) by increasing dietary Se from 0.3 to 3.0 mg/kg. Comparatively, expressions of *Gpx2*, *Gpx4*, *Dio3*, and *Sep15* were high in pituitary and *Dio1*, *Sepp1*, *Sephs2*, and *Gpx1* were high in liver. In conclusion, the mRNA abundances of the 12 selenoprotein genes in thyroid and pituitary of young pigs were resistant to dietary Se deficiency or excess. J. Nutr. 139: 1061–1066, 2009.

Introduction

Testis, thyroid, and pituitary are 3 major endocrine organs in the body that regulate growth, reproduction, and metabolism. The discovery of the impaired thyroid hormone metabolism in selenium (Se)-deficient animals (1,2) and the subsequent identification of deiodinases (3) signified the crucial roles of Se in endocrine functions. Although a number of studies have followed those initial experiments to explore Se biology in thyroid and other endocrine organs (for review, see 4,5), gene expressions of selenoproteins, especially the recently identified members, and their responses to dietary Se alterations remain largely unclear. Apparently, the limited availability of human samples and the small sizes of rodent samples represent one of the greatest challenges for any research of endocrine organs. In fact, pigs offer a greater physiological similarity and organ size than rodents as an excellent model of humans for this purpose (6,7).

Only 7 of the 24/25 selenoprotein genes (8) identified in mice and humans were cloned in pigs. These include *Gpx1*, *Gpx4*, *Dio1*, *Dio3*, *Txnrd1*, *Selk*, and *Sepw1*. There was no information on the other porcine selenoprotein genes except for the expressed sequence tag (EST)⁶ of *Gpx2*, *Sephs2*, *Sep15*, *Sepp1*, and *Sepp1*. Among these genes, *Gpx1*, *Gpx2*, and *Gpx4* code for 3 of the 5 Se-dependent glutathione peroxidases (GPX); *Dio1* and *Dio3* for 2 of the 3 iodothyronine deiodinases; and *Txnrd1* for 1 of the 3 thioredoxin reductases. *Sepp1* codes for selenoprotein P (SeP) that accounts for >50% of the Se in plasma (9). *Sepw1* codes for selenoprotein W that is highly expressed in muscle and brain (10). *Sephs2* codes for selenophosphate synthetase 2 that catalyzes the formation of mono-selenophosphate, the Se donor required for selenocysteine (Sec) synthesis (11). *Sep15* codes for selenoprotein 15kDa that is a redox protein associated with UDP-glucose:glycoprotein glucosyltransferase in endoplasmic reticulum and probably plays a role in the quality control of misfolded proteins (12). *Sepp1* codes for selenoprotein N that is associated with muscle diseases (13). *Selk* codes for selenoprotein K that is another redox protein

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³ Supplemental Figures 1–3 and Supplemental Tables 1–4 are available with the online posting of this paper at jn.nutrition.org.

* To whom correspondence should be addressed. E-mail: xl20@cornell.edu and jxia@isa.ac.cn.

⁶ Abbreviations used: BD, basal diet; CDS, coding sequence; Ct, cycle threshold; EST, expressed sequence tag; GPX, glutathione peroxidase; NCBI, National Center for Biotechnology Information; Q-PCR, real-time quantitative PCR; Sec, selenocysteine; SECIS, selenocysteine insertion sequence; SeP, selenoprotein P; SeMet, selenomethionine.

located in endoplasmic reticulum (14). Although characterizing the metabolic functions of all these selenoproteins will probably take a long time, the sequence homology analysis of the porcine EST database allows us to isolate the novel selenoprotein genes from pigs by in silico (computational) cloning followed by PCR and sequencing (15). The cloned sequence information will help us in designing primers to study regulation of these novel selenoprotein genes by dietary Se in pigs.

Previously, regulations of selenoprotein gene expression by dietary Se were determined mainly by using sodium selenite as the Se source and northern blot as the method of mRNA quantification. Data from those studies may present questionable physiological relevance and limited precision, because organic Se is the main source of Se in ordinary human diets (16,17) and northern blot provides only a semiquantitative estimate of mRNA abundances. Se-enriched yeast may be a better source of Se than sodium selenite (17) and real-time quantitative PCR (Q-PCR) is a more sensitive and accurate method for that type of research (18). Therefore, we conducted 2 experiments to: 1) clone *Gpx2*, *Sephs2*, *Sep15*, *Sepn1*, and *Sepp1* from pig tissues based on their candidate fragments from the porcine nucleotide database and characterize their sequence homology with the human genes; and 2) compare effects of 3 concentrations of dietary Se (as Se-enriched yeast) on mRNA levels of 12 selenoproteins assayed by Q-PCR, activities of 4 antioxidant enzymes, and Se concentrations in testis, thyroid, and pituitary with those in liver of young male pigs.

Materials and Methods

Experiment 1

Cloning of novel selenoprotein genes in porcine tissues. Based on the database of National Center for Biotechnology Information (NCBI), we selected *Gpx2*, *Sephs2*, *Sep15*, *Sepn1*, and *Sepp1* as the cloning targets in the present study. Data mining was conducted as previously described (19). Briefly, the mRNA (cDNA) or protein sequence of the human selenoprotein gene was used as the query sequence to search for highly similar sequences in pigs using the appropriate BLAST programs (NCBI). The acquired sequences in pig were assembled and extended using Vector NTI (Invitrogen, version 9.1.0). If the similar sequence(s) linked to UniGene database, more EST were available for assembling. The assembled gene was considered as a selenoprotein gene if the in-frame TGA for Sec was identified in the open reading frame and the Sec insertion sequence (SECIS) element in the 3'-untranslated region was identified by the online program SECISearch (8). The target porcine gene was amplified by RT-PCR. Primers (Supplemental Table 1) were designed based on homology analysis of the in silico cloned (presumed) selenoprotein gene sequence with the same gene from other species. If no similar porcine sequences were found by in silico cloning, sequences of the target gene from human, mouse, rat, and cattle were aligned to design degenerated primers in the highly conserved region. Total RNA was extracted using TRIzol (Invitrogen catalog no. 15596-026) from spleen (for *Sephs2* and *Sep15*), muscle (for *Sepn1*), small intestine (for *Gpx2*), and kidney (for *Sepp1*) of a Se-adequate, adult male Landrace pig. These tissues were selected as the RNA source for a given target gene based on the assumption that pigs and humans or mice share similar tissue mRNA abundance for the gene. The RT-PCR and the subsequent T/A cloning were carried out using commercial kits (TaKaRa, Dalian; reverse transcriptase, catalog no. D2620; DNA polymerase, catalog no. DR001AM, and pMD18-T vector catalog no. D101). A minimum of 3 positive transformants were sequenced for each gene (Invitrogen) and the sequence with the highest consensus was submitted to GenBank (NCBI).

Experiment 2

Pig, diet, and sample collection. Our protocol was approved by the Animal Care Office of Sichuan Agricultural University. A total of 30 weanling male pigs (3-wk old, PIC strain, Sichuan Lanyan Group) were

selected and fed a Se-deficient, corn-soybean meal (produced in the Se-deficient area in Sichuan, China) basal diet (BD) (Table 1) for 4 wk to adjust their Se status. The pigs were divided into 3 groups ($n = 10$) and fed the BD supplemented with 0, 0.3, or 3.0 mg Se/kg as Se-enriched yeast (Angel Yeast) for 8 wk. The analyzed Se concentrations in the experimental diets were 0.02, 0.30, and 3.05 mg/kg, respectively. At baseline and then biweekly, body weights were recorded for and blood samples collected from individual pigs. Plasma samples were prepared by centrifugation of the whole blood ($2200 \times g$; 15 min; 5804R Centrifuge, F45-30-11 rotor, Eppendorf) and stored at -80°C . At the end of the study, 6 pigs from each treatment group were killed to collect liver, testis, thyroid, and pituitary. After immediate dissection on an ice-cold surface, the organs were perfused with ice-cold isotonic saline, minced with surgical scissors, divided into aliquots, snap-frozen in liquid nitrogen, and stored at -80°C until use.

Concentrations of Se and activities of enzymes. The hydride generation-atomic fluorescence spectrometer (AFS-830, Titan Instruments) (20) was used to measure tissue and plasma Se concentrations against the standard Se reference [GBW (E) 080441, National Research Center for Certified Reference Materials, Beijing, China]. Tissue samples (0.2 g for pituitary and 0.5 g for other tissues) were thawed on ice and homogenized as previously described (21). Activities of GPX were measured by the coupled-method (22) (0.12 mmol/L of H_2O_2 as substrate) using an ultraviolet-visible spectrophotometer (DU800, Beckman). To assess the general antioxidant status of the experimental pigs, activities of glutathione reductase were measured using the method of Massey and Williams (23), activities of glutathione S-transferase were measured using a GST assay kit (A004, Nanjing Jiancheng Bioengineering Institute), and activities of superoxide dismutase were measured as described by Elstner and Heupel (24). Concentrations of protein were determined using the Bradford method (25).

Q-PCR analysis of the selenoprotein mRNA abundance. Primers (Supplemental Table 2) for the 12 selenoprotein genes (5 of them were cloned in this study) and 2 reference genes, β -actin gene (*Actb*) and glyceraldehyde 3-phosphate dehydrogenase gene (*Gapdh*), were designed using Primer Express 3.0 (Applied Biosystems). The total RNA was extracted as described above. Potential DNA contamination of the extraction was eliminated using the DNA-free kit (Ambion, catalog no. AM1906) and the RNA quality was verified by both agarose gel (1%) electrophoresis and spectrometry (A260/A280). The mRNA levels of selenoprotein genes were analyzed using Q-PCR (ABI 7900HT, Applied

TABLE 1 Composition of BD (as fed)

Ingredients ¹	Content, g/kg
Corn	688.2
Soybean meal	280.0
Choline	1.0
L-Lysine	4.0
DL-Methionine	0.5
L-Tryptophan	0.3
L-Threonine	1.5
Salt	3.0
CaHPO ₄	7.0
CaCO ₃	4.0
Trace mineral premix ²	10.0
Vitamin premix ³	0.5

¹ The Se concentrations of corn, soybean, and Se-rich yeast (Angel Yeast) were 0.005, 0.03, and 1000 mg/kg. Analyzed Se concentration in the BD was 0.02 mg/kg.

² Trace mineral premix provided per kg diet: FeSO₄·7H₂O, 993 mg; CuSO₄·5H₂O, 786 mg; ZnSO₄·7H₂O, 440 mg; MnSO₄, 27.5 mg; KI, 0.4 mg; and colistin sulfate, 40 mg.

³ Vitamin premix provided per kg diet: retinyl acetate, 3027 μg ; cholecalciferol, 22 μg ; *d*- α -tocopheryl acetate, 32 mg; menadione, 2 mg; thiamin, 4 mg; riboflavin, 14 mg; calcium pantothenate, 40 mg; niacin, 60 mg; pyridoxol, 6 mg; *d*-biotin, 0.2 mg; folacin, 1.2 mg; and cobalamin, 72 μg .

Biosystems). Briefly, RT and PCR amplifications were conducted in duplicates for both target and reference genes. Negative controls containing the template RNA and all PCR reagents, but not reverse transcriptase, were included to determine the RNA purity from DNA contamination. The reaction mixture (6.0 μ L) contained 3.0 μ L of freshly premixed 2 \times QuantiTect SYBR Green RT-PCR Master mix and QuantiTect RT mix (QIAGEN, catalog no. 204243), 0.4 μ mol/L of the primer pair, and 100 ng of RNA template. The PCR consisted of 1 cycle of 48°C for 30 min, 1 cycle of 95°C for 10 min, and 40 cycles of 95°C for 15 s and 58°C for 1 min, followed by the dissociation step at 95°C for 15 s, 60°C for 20 s, and 95°C for 15 s. To confirm the specific amplification, melt curve analysis was performed (Supplemental Fig. 1) and the products were also visualized on ethidium bromide-stained 2% (wt:v) agarose gel after electrophoresis using Tris-acetate-EDTA buffer.

Relative mRNA abundances of the 12 selenoprotein genes in the 4 tissue samples were determined using the Δ cycle threshold (Δ Ct) method as outlined in the protocol of Applied Biosystems. In brief, a Δ Ct value was the Ct difference between the target selenoprotein gene and the reference gene (Δ Ct = Ct^{target} - Ct^{reference}). *Actb* was used as the reference gene and its reliability was confirmed by the perfect parallelism of its Ct values in all the tissue samples with those of *Gapdh* (Supplemental Fig. 2). For each of the target selenoprotein genes, the $\Delta\Delta$ Ct values of all the samples were then calculated by subtracting the average Δ Ct of the liver from the Δ Ct of all the other tissue samples. The $\Delta\Delta$ Ct values were converted to fold differences by raising 2 to the power $-\Delta\Delta$ Ct ($2^{-\Delta\Delta$ Ct}).

Statistical analysis. One-way ANOVA followed by Bonferroni *t* test (SPSS for Windows 13.0) was used to test the effects of the 3 dietary Se levels on each variable within the same tissue. Two-way ANOVA was used to determine the tissue-specific distribution for a given gene at the same dietary Se level. Data are presented as means \pm SE. The null hypothesis was rejected if $P < 0.05$ (2-tailed).

Results

Experiment 1

Porcine *Gpx2*, *Sephs2*, *Sep15*, *Sepr1*, and *Sepp1* were cloned and their sequences were submitted to NCBI (Table 2). Based on the secondary structures of their SECIS elements (Supplemental Fig. 3), *Gpx2*, *Sephs2*, and *Sepr1* belong to form 1, whereas *Sep15* and *Sepp1* belong to form 2 (26). Among the 5 novel genes, the number of base pairs in the coding sequence (CDS) was 573 for *Gpx2*, 1356 for *Sephs2*, 489 for *Sep15*, and 1173 for *Sepp1*. The incomplete *Sepr1* CDS had 1498 base pairs and lacked ~180 base pairs in the 5'-site. There were 14 in-frame TGA codons in the porcine *Sepp1*, but only 1 in each of the other

TABLE 2 Characteristics of the 5 novel pig selenoprotein genes

Gene	Accession no.	Length		Identity to human		Sec position ¹
		CDS	Peptide	CDS	Peptide	
		<i>bp</i>	<i>amino acids</i>	—%—		
<i>Gpx2</i>	DQ898282	573	190	94	97	40
<i>Sephs2</i>	EF033624	1356	451	89	92	63
<i>Sep15</i>	EF178474	489	162	85	93	93
<i>Sepr1</i> ²	EF113595	1498	498	93	93	370
<i>Sepp1</i>	EF113596	1173	390	84	76	14 Sec ³

¹ From the N terminus.

² About 180 bp in the 5'-site of CDS of porcine *Sepr1* was not available, which in turn resulted in the absence of ~60 amino acids in the N terminus of the protein sequence. The missing counterpart in the CDS or peptide of human *Sepr1* was not compared for the identity analysis.

³ Positions of the 14 Sec in the *Sepp1* peptide were at the 59, 267, 286, 309, 311, 327, 339, 352, 354, 361, 376, 378, 385, and 387 residues.

4 novel genes. Between pigs and humans, the CDS homology ranged from 84 to 94% and the protein sequence homology was $\geq 92\%$ for all the genes except for *Sepp1* (Table 2).

Experiment 2

Growth performance, tissue Se concentration, and enzyme activity. Pigs fed the 3 dietary Se concentrations had similar growth performance (Supplemental Table 3). Plasma Se concentrations of pigs were increased ($n = 6$; $P < 0.05$) by 0.3 and 3.0 mg Se/kg relative to the Se-deficient pigs (0.02 mg Se/kg) at the end of study, whereas GPX3 activities were sigmoidally raised (Table 3). Compared with 0.3 mg Se/kg, dietary Se deficiency decreased ($P < 0.05$), whereas dietary Se excess increased ($P < 0.05$) the Se concentrations in all 4 tissues. Liver had the greatest relative (19% to 3-fold) fluctuation in Se concentration between 0.02 and 3.0 mg Se/kg among the 4 tissues. Likewise, dietary Se deficiency decreased ($P < 0.05$) GPX activity by 95% in liver but only 32% in pituitary. However, pigs fed 3.0 mg Se/kg had similar (43–88%) increases in GPX activity among all 4 tissues relative to those of pigs fed 0.3 mg Se/kg. Dietary Se concentrations did not affect activities of the other 3 antioxidant enzymes in any of the 4 tissues (Supplemental Table 4).

Abundances of selenoprotein mRNA. Compared with pigs fed 0.3 and/or 3.0 mg Se/kg, those fed the BD had lower ($P < 0.05$) mRNA levels of *Gpx1*, *Sepw1*, *Sepr1*, and *Txnrd1* (Fig. 1A–D) in liver. In contrast, pigs fed 3.0 mg Se/kg had lower ($P < 0.05$) mRNA levels of *Txnrd1* and *Sep15* in testis than those fed 0.3 mg Se/kg (Fig. 1D,E). Dietary Se did not affect the mRNA levels of any gene in thyroid and pituitary or *Gpx2*, *Gpx4*, *Dio3*, *Selk*, *Dio1*, *Sepp1*, and *Sephs2* in liver and testis (Figs. 2 and 3). Pituitary had higher ($P < 0.05$) mRNA levels of *Gpx2*, *Gpx4*, *Dio3*, and *Sep15* than the other 3 tissues at all 3 dietary Se concentrations. Pituitary also had higher ($P < 0.05$) mRNA levels of *Selk* than testis and thyroid at dietary Se deficiency. Compared with the endocrine tissues, liver had higher ($P < 0.05$) mRNA levels of *Dio1*, *Sepp1*, *Sephs2*, and

TABLE 3 Tissue Se concentration and GPX activity of pigs fed diets containing 0, 0.3, or 3.0 mg Se/kg for 8 wk¹

Measures	BD	BD + 0.3 mg Se/kg	BD + 3.0 mg Se/kg
Se, μ mol/L plasma or nmol/g tissue			
Plasma			
Initial (wk 0)	0.61 \pm 0.04	0.73 \pm 0.04	0.68 \pm 0.08
Final (wk 8)	0.29 \pm 0.04 ^a	1.42 \pm 0.10 ^b	2.45 \pm 0.12 ^c
Liver	1.03 \pm 0.13 ^a	5.46 \pm 0.34 ^b	16.40 \pm 1.02 ^c
Testis	1.48 \pm 0.16 ^a	2.59 \pm 0.22 ^b	4.60 \pm 0.23 ^c
Thyroid	0.57 \pm 0.04 ^a	1.85 \pm 0.08 ^b	5.22 \pm 0.11 ^c
Pituitary ²	3.10	4.35	6.07
GPX, EU/mg protein			
Plasma			
Initial (wk 0)	15.24 \pm 1.45	16.62 \pm 1.00	19.50 \pm 1.85
Final (wk 8)	3.96 \pm 1.05 ^a	13.26 \pm 1.08 ^b	14.38 \pm 1.70 ^b
Liver	7.96 \pm 1.59 ^a	168.77 \pm 19.27 ^b	282.84 \pm 11.75 ^c
Testis	15.23 \pm 0.91 ^a	25.27 \pm 3.85 ^{a,b}	36.08 \pm 3.64 ^b
Thyroid	1.83 \pm 0.21 ^a	11.43 \pm 1.64 ^b	21.41 \pm 1.60 ^c
Pituitary	18.03 \pm 1.45 ^a	26.32 \pm 3.98 ^a	45.90 \pm 2.30 ^b

¹ Values are means \pm SE, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

² Samples were pooled by group due to limited amounts of tissue.

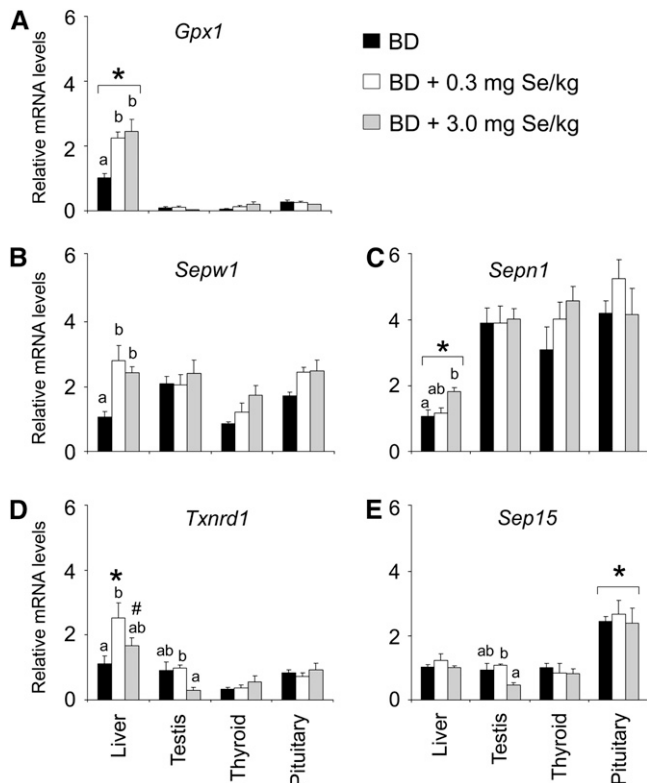


FIGURE 1 Abundance of *Gpx1*, *Sepw1*, *Sepn1*, *Txnrd1*, and *Sep15* mRNA in tissues of pigs fed 0, 0.3, or 3.0 mg Se/kg diet for 8 wk. Data are means \pm SE, $n = 4$. Within a tissue, labeled means without a common letter differ, $P < 0.05$. *Different from other tissues at that Se concentration, $P < 0.05$. #Different from corresponding testis and thyroid, $P < 0.05$.

Gpx1 at all 3 dietary Se concentrations and higher ($P < 0.05$) mRNA levels of *Txnrd1* at 0.3 mg Se/kg.

Discussion

All 5 cloned novel porcine selenoprotein genes, *Gpx2*, *Sephs2*, *Sep15*, *Sepn1*, and *Sepp1*, bear the 2 identifications of selenoproteins: in-frame TGA (UGA) codon and SECIS element in the 3'- untranslated region (8,26). Like their homologs reported in other mammals, *Gpx2*, *Sephs2*, *Sep15*, and *Sepn1* code respective selenoproteins with 1 Sec. In contrast, the porcine *Sepp1* mRNA (cDNA) contains 14 Sec codons and 11 of them appeared in the last 100 amino acid residues. This number of Sec is within the range of 10 to 17 in SelP characterized from other species (27) and their location in the protein is consistent with the fact that the C-terminal, Se-rich domain of SelP is critical for the maintenance of Se in brain and testis (28). The SECIS elements of the 5 pig selenoprotein genes, like other eukaryotic SECIS elements, fall into 2 classes of structural models: form 1 and form 2 (26). Compared with the human homologs, the DNA sequences in the CDS of the 5 porcine selenoproteins had a homology of 84–94% and the deduced amino acid sequences had a homology $\geq 92\%$, except for *Sepp1*. Thus, our success in cloning the 5 novel porcine selenoprotein genes and in demonstrating their high sequence homology to the human homologs provides molecular justification for the use of pigs as a human model to study Se biology in endocrine tissues.

The results of Expt. 2 show similar responses of Se distribution and GPX activity to dietary Se between liver and the 3

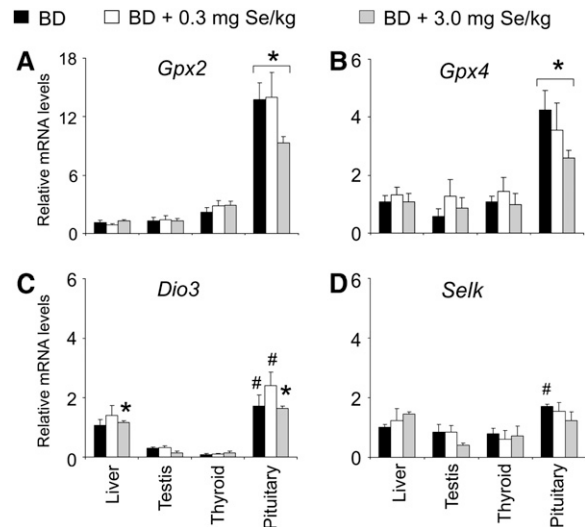


FIGURE 2 Abundance of *Gpx2*, *Gpx4*, *Dio3*, and *Selk* mRNA in tissues of pigs fed 0, 0.3, or 3.0 mg Se/kg for 8 wk. Data are means \pm SE, $n = 4$. *Different from other tissues at that Se concentration, $P < 0.05$. #Different from corresponding testis and thyroid, $P < 0.05$.

endocrine tissues. Although dietary Se deficiency downregulated mRNA levels of *Gpx1*, *Sepw1*, *Sepn1*, and *Txnrd1* in liver, mRNA abundance of the 12 selenoprotein genes in thyroid and pituitary remained fairly constant across all 3 dietary Se concentrations. Probably due to a relative large variation, a potential downregulation of the *Sepp1* mRNA abundance in liver by Se deficiency was not significant ($P = 0.23$). The downregulation of *Gpx1* mRNA in the Se-deficient liver may be explained by the low rank of GPX1 in the hierarchy of selenoproteins in competing for Se (21,29–33) and the activation of nonsense-mediated decay under limited Se supply (33,34). The decreased hepatic mRNA levels of the other 3 selenoprotein genes in the Se-deficient pigs were also similar to those reported in other species, although their changes were somewhat less than those of *Gpx1* (21,29,32,35,36). Whereas the hepatic *Gpx4*

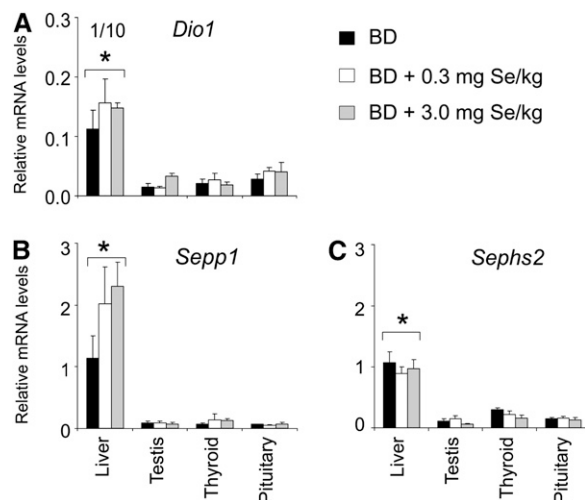


FIGURE 3 Abundance of *Dio1*, *Sepp1*, and *Sephs2* mRNA in tissues of pigs fed 0, 0.3, or 3.0 mg Se/kg for 8 wk. Data are means \pm SE, $n = 4$. Values for *Dio1* mRNA abundances in liver were minimized to 10% of the originals, indicated by "1/10." *Different from other tissues at that Se concentration, $P < 0.05$.

mRNA remained unchanged by dietary Se in the present study, it was actually decreased by Se deficiency in a previous study in which pigs were fed sodium selenite and mRNA was detected using northern blot with a rat cDNA probe (30). The lack of decrease in mRNA abundances of *Gpx1* and other selenoprotein genes in the 3 Se-deficient endocrine tissues implies at least 2 possibilities. First, the endocrine tissues rank higher than liver in the hierarchy of Se metabolism. Second, the nonsense-mediated decay of *Gpx1* and other selenoprotein genes may not occur in the endocrine tissues. It will be interesting to find out how these 2 possibilities affect the overall control of Se metabolism and endocrine functions under various physiological conditions (37).

The downregulation of *Txnrd1* and *Sep15* mRNA levels in testis of pigs fed 3.0 mg Se/kg, compared with those fed 0.3 mg Se/kg, appears to be unique. Because these declines were not observed in the other 3 tissues, the expression of selenoprotein genes in testis seems to be more sensitive to the high level of dietary Se. As *Txnrd1* and *Sep15* code for antioxidant enzyme or protein that may control the quality of cellular proteins (12,38), downregulation of these genes in testis may be an outcome of Se toxicity (39–41) or help precipitate the toxicity. As shown in mice (42), pigs had higher hepatic mRNA abundances of *Gpx1*, *Txnrd1*, *Dio1*, *Sepp1*, and *Sephs2* than those in the endocrine tissues. The higher *Dio1* mRNA in liver than in thyroid helps explain why the hepatic iodothyronine deiodinase 1 is the most important in catalyzing the circulating thyroxine to the more active 3,5,3'-tri-iodothyronine, although thyroxine is produced and released from thyroid (2). Similarly, the high hepatic *Sepp1* mRNA level is consistent with the fact that liver is the major source of plasma SelP (43). However, another group reported that testis had higher mRNA levels of *Txnrd1* and *Sepp1* than liver in rats (44). It is unclear if the discrepancy from our finding was due to species (rats vs. pigs), source of Se (sodium selenite vs. Se yeast), or detection method (ribonuclease protection analysis vs. Q-PCR). Notably, pituitary had higher mRNA levels of *Gpx2*, *Gpx4*, *Dio3*, and *Sep15* than liver and the other 2 endocrine tissues. The abundant gene expression of these selenoproteins and their resistance to dietary or tissue Se alteration raise a question as to how these selenoproteins affect the pituitary endocrine function (5). Previously, *Gpx2* mRNA expression was detected only in the gastrointestinal tissues and liver by northern blot (45). It will be interesting to discover the relative contribution of GPX2, along with GPX4, to the total GPX activity in pituitary. Expression of *Sepn1* mRNA was higher in the 3 endocrine tissues than in liver. Being involved in a genetic muscular disorder and the early development and cell proliferation or regeneration (13), the production and function of selenoprotein N in endocrine tissues warrants more research.

Different from the dietary Se regulation of *Gpx1* mRNA (29,31,44), we have observed 43–88% increases ($P < 0.05$) in GPX activity among all 4 tissues by elevating dietary Se from 0.3 to 3 mg/kg. This upregulation of tissue GPX1 activity by excess dietary Se is also different from the saturation of GPX1 activity in liver of rats or GPX3 activity in the plasma or serum of rats (31,32,46,47). In a previous pig study, GPX1 activity in thyroid and pituitary was not elevated by increasing dietary Se supplementation from 0.1 to 0.3 mg/kg (30). The discrepancy from the present study may be related to supplemental Se forms (organic vs. inorganic) and the interval of dietary Se concentrations (from 0.3 to 3.0 mg Se/kg vs. 0.1 to 0.3 mg Se/kg). Presumably, Se in the Se-enriched yeast exists mainly in the organic form such as selenomethionine (SeMet). When animals ingest this type of Se, their plasma and tissue Se concentrations contain Se present in selenoproteins and in randomly incorporated SeMet. When Se is

transported out of liver into blood, the plasma GPX activity (assayed) and the SelP concentration (not assayed herein) reach plateaus. Then, the SeMet-derived Se probably plays a major role in continuously elevating the plasma Se concentration, because Se in small molecular forms counts for <3% of the total (48). This complicates the comparison of Se concentrations and GPX activities. Nevertheless, the concomitant elevations of Se concentration and GPX activity in tissues, particular in endocrine tissues, to the super-nutritional level of dietary Se supplementation may offer a different perspective for the antitumor mechanism of dietary Se (49). In addition, the lack of responses of mRNA levels of *Gpx1* and other selenoprotein genes in any of the tissues to dietary Se increases from 0.3 to 3 mg/kg suggests limitation of these indicators to assess body Se status (50) when Se supply exceeds the requirement.

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