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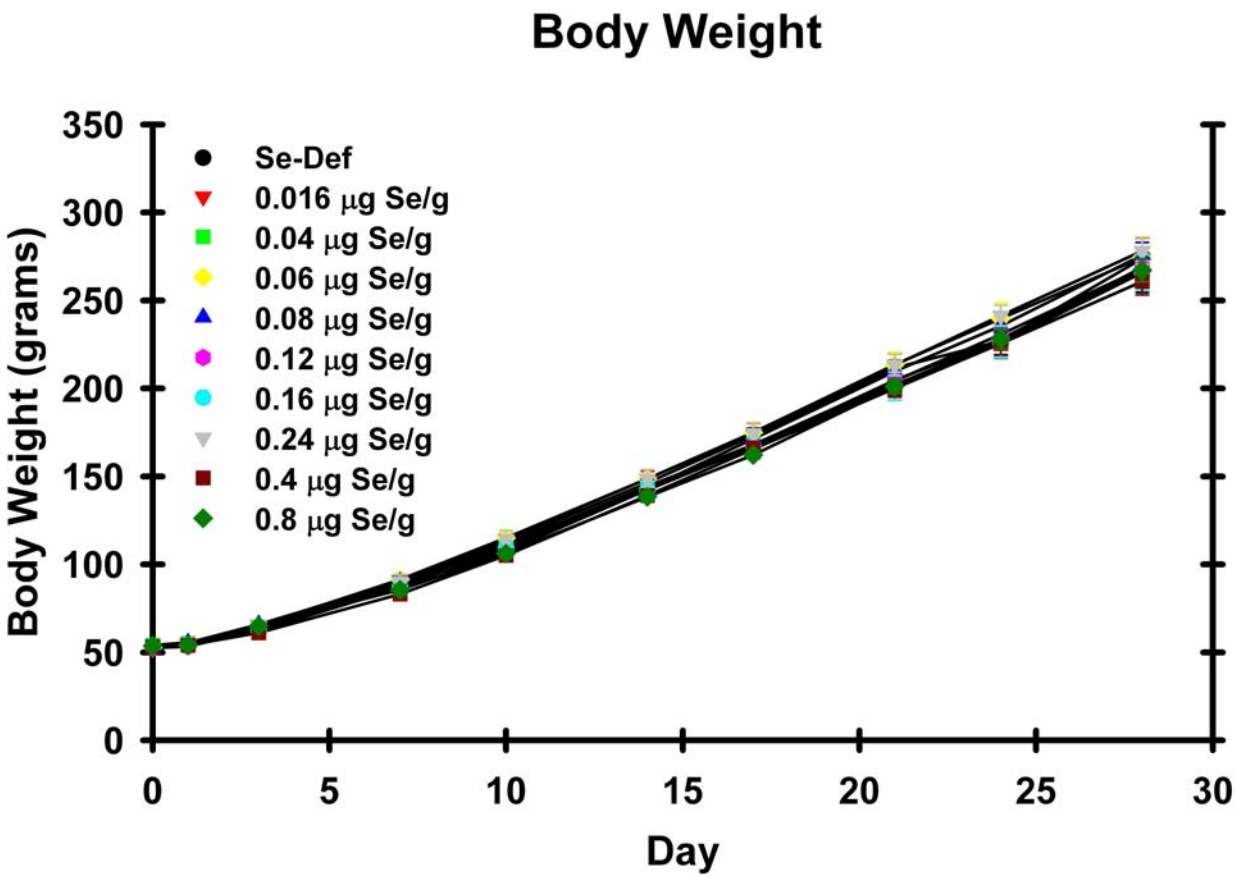
Barnes et al., Supplemental Figure 1



SUPPLEMENTAL FIGURE 1 Effect of dietary Se on rat body weight. Male weanling rats were supplemented with Se at the indicated levels for 28 d (n = 5 or 6/diet) and weighed biweekly. Values are the mean weights ± SEM. There were no significant effects (*P* > 0.05) of dietary Se level at any timepoint.

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SUPPLEMENTAL TABLE 1 Selenoprotein genes and qRT-PCR primers for rat selenoproteins

Gene[1]	Accession[2]	Gene Name	Forward Primer[3]	Reverse Primer[3]
Actb	NM_031144	Actin, beta	5'-cctgggtatggaatcctgtg	5'-cttctgcatcctgtcagcaa
Dio1	NM_021653	Deiodinase, iodothyronine, type I	5'-gcctctcaggacagaagtgc	5'-gtcagctgtggaggcaaagt
Dio2	NM_031720	Deiodinase, iodothyronine, type II	5'-ggactacgctgtgtctggaa	5'-ctgcacaggcaaagtcaaga
Dio3	NM_017210	Deiodinase, iodothyronine, type III	5'-ctgtgctctggttctggaca	5'-cgcaactcagacacctggta
Gapdh	NM_017008	Glyceraldehyde-3-phosphate dehydrogenase	5'-ccatcaccatcttccaggag	5'-cggagatgatgaccctttg
Gpx1	NM_030826	Glutathione peroxidase 1	5'-gctgctcattgagaatgtcg	5'-gaatctcttcattcttgccatt
Gpx2	NM_183403	Glutathione peroxidase 2	5'-cctagtggttctcggcttcc	5'-tgcccattgacatcacactt
Gpx3	NM_022525	Glutathione peroxidase 3	5'-cgagtatggagcccttacca	5'-aatgggccaagttcttcttg
Gpx4	NM_017165	Glutathione peroxidase 4	5'-ccggctacaatgtcaggttt	5'-acgcagccgttcttatcaat
Selh	NM_001114939	Selenoprotein H	5'-aactggaggccccagagata	5'-ggctcaggaaatttgagcttt
Seli	XM_343031	Selenoprotein I	5'-tcactgctgccttcactctg	5'-ggaccgatactcttctcttcca
Selk	NM_207589	Selenoprotein K	5'-aaccggaggaaagatggttt	5'-ccccgtagcctcttcttttc
Selm	XM_001115013	Selenoprotein M	5'-aaggaggtgaaggcctttgt	5'-tcatttggctgagtgggattt
Sepn1	XM_342942	Selenoprotein N	5'-gttcaccggccatatcatct	5'-catgttgctggtctcactgg
Selo	NM_001085485	Selenoprotein O	5'-ctcattggcactcaagcaaa	5'-tccttgtccagacgctctct
Sepp1 (SelP)	NM_019192	Selenoprotein P	5'-tccttcctcactttcccgta	5'-tctgagggctctgtggtttt
Sepx1 (MsrB1)	NM_001044285	Methionine-R-sulfoxide reductase	5'-aagtgcggctatgagctgtt	5'-acttgccacaggacaccttt
Sels	NM_173120	Selenoprotein S	5'-cttcagctgcgtccttctct	5'-ctgcattctcaaacgagcag
Selt	NM_001014253	Selenoprotein T	5'-cgtgcccagcaagagatta	5'-tcaatgcggatgtctggata
Selv	XR_009209	Selenoprotein V	5'-cccagcacagaacttcgttt	5'-tgatgctccagggtcttttt
Sepw1	NM_013027	Selenoprotein W	5'-gcccaagtatctccagctca	5'-ttccggaacttgctctctgt
Sep15	NM_133297	Selenoprotein 15	5'-ctgcgatctccttggacagt	5'-ggaacctccccaattttcat
Sephs2 (SPS2)	NM_001079889	Selenophosphate synthetase 2	5'-actcagtgtgagccagagca	5'-cccaccgatgataatccaag
Txnrd1	NM_031614	Thioredoxin reductase 1	5'-ggcctcgacgtcactgtaat	5'-tccctgcttcaatctgttca
Txnrd2	NM_022584	Thioredoxin reductase 2	5'-cgctggagaagtcacacaag	5'-cagtaggatccaggccagag
Txnrd3	NM_001106609	Thioredoxin reductase 3	5'-tgaagtcacacaggggtttg	5'-gtaatgtccagccctgagga

[1]Gene symbol with common alternative symbol given in parentheses.
[2]Accession number from NCBI Entrez Gene database.
[3]Forward and reverse primers used to amplify ~150 bp qRT-PCR product that span an intron.

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SUPPLEMENTAL TABLE 2 Effect of dietary selenium on tissue selenium concentration and selenoenzyme activity in rats (Expt. 2)[1].

Biomarker	Dietary Se (µg Se/g diet) 0	0.016	0.04	0.06	0.08	0.12	0.16	0.24	0.4	0.8	ANOVA *P*-value
					µmol Se/g wet weight						
Liver Se[2]	0.20±0.01^{a}	0.73±0.05^{a}	2.50±0.20^{ab}	4.58±0.34^{bc}	6.31±0.29^{cd}	7.44±0.51^{de}	7.56±0.53^{de}	7.03±0.36^{cd}	10.13±0.23^{ef}	12.62±0.96^{f}	<0.0001
Kidney Se[2]	1.56±0.15^{a}	3.38±0.19^{ab}	6.74±0.64^{abc}	7.63±0.44^{bcd}	9.33±0.05^{cd}	11.54±0.32^{cde}	11.42±0.71^{cde}	13.13±0.51^{de}	15.63±0.44^{ef}	19.24±2.42^{f}	<0.0001
					EU/g protein						
Plasma Gpx3[2]	1.9±0.2^{a}	17.9±1.2^{a}	52.6±2.3^{b}	74.2±3.6^{c}	78.3±4.7^{cd}	88.9±3.9^{cde}	95.6±2.8^{cde}	100.7±4.0^{e}	102.8±4.3^{e}	99.0±4.2^{de}	<0.0001
RBC Gpx1[2]	63.3±1.7^{a}	88.0±2.7^{ab}	130.4±4.2^{bc}	172.9±5.9^{cd}	219.6±4.7^{de}	222.8±11.9^{e}	260.8±4.3^{ef}	290.5±10.6^{f}	342.3±12.4^{g}	434.1±11.8^{h}	<0.0001
Liver Gpx1[2]	14.9±2.2^{a}	19.8±1.9^{a}	186.5±22.4^{a}	503.3±44.3^{b}	661.4±11.4^{bc}	834.0±53.3^{cd}	898.7±28.7^{d}	819.2±33.6^{cd}	930.4±56.1^{d}	818.4±43.0^{cd}	<0.0001
Kidney Gpx1[2]	23.7±1.5^{a}	54.8±3.6^{a}	158.3±8.2^{b}	245.3±8.3^{c}	322.9±14.9^{c}	425.9±19.8^{d}	477.0±13.1^{d}	490.8±20.9^{d}	500.3±16.6^{d}	497.1±5.2^{d}	<0.0001
Muscle Gpx1[2]	5.2±0.9^{a}	9.5±1.1^{a}	21.7±1.4^{ab}	48.3±4.5^{bc}	51.9±4.0^{bc}	73.1±6.6^{cd}	68.3±6.1^{cd}	85.0±5.7^{cde}	98.8±11.1^{de}	116.0±10.7^{e}	<0.0001
Liver Gpx4	4.7±0.7^{a}	4.4±0.5^{a}	7.0±0.4^{b}	8.4±0.4^{bc}	9.2±0.4^{cd}	10.6±0.6^{d}	9.9±0.6^{cd}	9.5±0.4^{cd}	9.3±0.6^{cd}	10.5±0.6^{d}	<0.0001
Kidney Gpx4[2]	6.6±1.5^{ab}	5.7±0.2^{a}	9.0±0.1^{abc}	11.3±0.5^{bcd}	12.7±0.3^{cd}	14.1±0.8^{d}	12.0±1.7^{cd}	14.0±0.5^{d}	13.0±0.5^{cd}	14.3±0.5^{d}	<0.0001
Muscle Gpx4	3.0±0.3	2.3±1.1	2.6±0.3	2.8±0.9	2.8±0.3	3.3±1.0	2.6±0.5	2.3±0.4	3.3±0.8	3.0±0.3	0.832
Liver Trxrd[2]	2.1±0.2^{a}	6.7±0.5^{a}	20.2±0.7^{b}	26.9±1.6^{c}	29.5±0.9^{cd}	31.1±0.6^{cd}	30.9±0.8^{cd}	30.8±1.1^{cd}	34.1±0.9^{d}	34.7±1.0^{d}	<0.0001

[1]Values are presented as mean±SEM, n = 5 or 6/diet, except for liver Se, kidney Se, and muscle GPX4, n = 3/diet. Means in a row with superscripts without a common letter differ significantly, $P < 0.05$.
[3]Differences determined by Scheffé's test as variances were not homogeneous.

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SUPPLEMENTAL TABLE 3 Effect of dietary selenium on selenoprotein mRNA abundance in rats (Expt. 1) [1].

Tissue/ Gene	Dietary Se (µg Se/g diet) 0	0.02	0.05	0.075	0.1	0.15	0.2	0.3	ANOVA *P*-value
					%[2]				
Liver									
Gpx1	12.2±1.0[a]	30.9±5.2[b]	65.7±3.7[c]	97.1±3.1[d]	103.8±2.4[d]	105.4±10.4[d]	100.0±5.0[d]	97.9±4.4[d]	< 0.0001
Gpx4	76.0±6.4	82.4±5.2	79.9±7.4	87.2±9.8	93.4±7.2	90.3±5.8	100.3±7.4	84.9±7.9	0.38
Selh	30.6±1.4[a]	42.2±1.6[a]	66.2±6.8[b]	80.9±2.0[bc]	83.0±4.7[bc]	92.8±13.8[c]	100.2±6.8[c]	93.0±7.2[c]	< 0.0001
Selk	40.9±3.6[a]	65.7±1.5[b]	79.6±7.1[bc]	93.1±8.8[c]	84.5±10.4[bc]	88.1±5.0[c]	99.8±1.4[c]	84.6±4.0[bc]	0.0001
Selm	84.3±2.9[a]	87.1±1.1[ab]	86.1±5.0[ab]	115.2±9.7[c]	103.4±7.8[bc]	111.2±3.8[c]	100.1±5.7[abc]	88.8±1.5[ab]	0.004
Sepp1	61.6±5.1[a]	72.9±8.5[ab]	75.5±3.1[abc]	96.4±1.8[d]	89.8±3.0[cd]	89.6±1.9[cd]	99.8±2.3[d]	84.3±7.5[bcd]	0.0005
Sepw1[3]	18.6±1.6[a]	42.1±1.0[ab]	60.1±3.8[bc]	77.6±1.2[bcd]	76.2±4.5[bcd]	110.0±12.1[d]	100.6±8.7[d]	80.8±3.8[cd]	< 0.0001
Txnrd1	60.9±1.2[a]	67.2±2.9[ab]	80.4±7.5[bc]	108.1±8.5[d]	103.4±4.7[d]	94.6±2.9[cd]	100.2±6.0[d]	95.2±8.8[cd]	0.0002
Txnrd2	59.7±1.7	82.9±8.2	91.7±16.1	97.9±3.0	93.8±6.8	88.8±10.5	101.8±14.7	92.2±12.4	0.22
Kidney									
Gpx1	33.9±1.8[a]	55.2±3.3[ab]	74.8±8.1[bc]	92.1±8.1[c]	95.5±10.0[c]	93.4±9.1[c]	102.0±16.2[c]	95.2±11.6[c]	0.0011
Gpx3	57.9±5.6	80.6±6.8	71.7±8.6	108.2±19.4	105.8±13.0	105.5±7.6	103.1±19.8	102.9±22.3	0.16
Gpx4	126.8±7.2	110.8±14.6	122.6±7.8	96.8±12.6	97.7±8.8	102.6±14.3	103.4±20.6	113.3±13.8	0.66
Selh	53.3±8.4[a]	74.1±4.2[ab]	96.3±2.2[bc]	88.3±16.8[bc]	105.3±9.6[bc]	113.7±9.8[c]	101.0±12.3[bc]	108.8±4.7[c]	0.007
Selm	98.8±10.7	95.7±9.0	109.2±8.9	103.6±5.4	109.6±18.8	101.2±10.0	102.1±16.2	99.8±14.4	0.99
Sepw1	41.3±4.6[a]	59.7±4.7[ab]	84.5±7.3[bc]	99.4±7.0[c]	89.7±3.0[c]	91.1±5.6[c]	102.6±18.1[c]	99.4±9.8[c]	0.001
Sephs2[3]	134.0±4.1[b]	117.8±5.1[ab]	124.5±11.3[ab]	98.6±11.8[ab]	86.1±4.9[a]	91.2±0.5[a]	99.7±2.3[ab]	81.1±2.9[a]	0.0006

[1]Values are presented as mean±SEM, n = 3/diet. Means in a row with superscripts without a common letter differ significantly, $P < 0.05$.
[2]Relative selenoprotein mRNA values determined by qRT-PCR, determined in triplicate for each sample of total RNA isolated from the indicated tissue, and normalized to the average of Gapdh and Actb mRNA in the same sample, and expressed as a percentage of the levels in Se-adequate (0.2 µg Se/g diet) rats.
[3]Differences determined by Scheffé's test as variances were not homogeneous.

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SUPPLEMENTAL TABLE 4 Effect of dietary selenium on selenoprotein mRNA abundance in rats (Expt. 2)[1].

Tissue/ Gene	Dietary Se (µg Se/g diet) 0	0.016	0.04	0.06	0.08	0.12	0.16	0.24	0.4	0.8	ANOVA *P*-value
					%[2]						
Liver											
Dio1	46.6±8.3^{a}	65.2±6.5^{ab}	78.6±4.4^{bcd}	74.2±4.9^{bc}	86.0±6.5^{bcd}	97.1±3.9^{cde}	93.9±0.5^{cde}	113.3±4.1^{e}	85.2±7.8^{bcd}	99.4±16.1^{de}	0.0002
Gpx1[3]	9.5±1.0^{a}	20.3±4.7^{a}	51.6±13.8^{ab}	83.5±12.2^{bc}	88.6±3.1^{bc}	90.6±6.4^{bc}	87.2±1.8^{bc}	96.8±8.3^{bc}	111.9±1.7^{c}	98.8±4.3^{c}	<0.0001
Gpx3	34.9±2.9^{a}	56.3±8.7^{ab}	84.5±14.1^{bc}	80.2±3.2^{bc}	85.9±8.3^{bc}	84.2±1.9^{bc}	106.2±16.6^{c}	108.6±16.4^{c}	112.1±20.3^{c}	80.9±9.8^{bc}	0.005
Selh	21.3±2.8^{a}	34.9±4.5^{a}	71.5±12.3^{b}	85.0±6.7^{bcd}	94.4±9.2^{bcd}	99.9±4.6^{cd}	75.1±9.6^{bc}	99.4±5.1^{cd}	108.2±5.9^{d}	104.9±12.4^{d}	<0.0001
Selk	25.2±6.9^{a}	50.3±9.0^{a}	80.1±18.7^{b}	102.9±5.5^{b}	94.6±3.3^{b}	100.4±6.7^{b}	91.6±4.8^{b}	101.8±8.9^{b}	99.6±6.8^{b}	100.0±9.2^{b}	<0.0001
Sepn1	53.8±9.2^{a}	73.3±8.6^{ab}	85.8±6.0^{bc}	97.6±3.0^{bc}	102.1±8.2^{c}	96.4±8.0^{bc}	102.1±10.4^{c}	106.1±4.1^{c}	98.2±11.2^{bc}	93.7±11.0^{c}	0.007
Sepp1	52.5±7.1^{a}	69.3±6.6^{ab}	80.4±7.9^{bc}	100.0±9.4^{c}	98.5±6.3^{c}	102.4±2.5^{c}	101.5±5.7^{c}	84.7±11.0^{bc}	102.5±1.7^{c}	103.1±10.6^{c}	0.0008
Selt	43.8±4.8^{a}	67.7±13.7^{ab}	84.3±22.0^{b}	93.2±10.2^{b}	100.7±12.3^{b}	101.7±15.4^{b}	86.0±11.0^{b}	100.1±1.6^{b}	104.3±10.3^{b}	99.4±6.8^{b}	0.04
Sepw1	16.0±1.9^{a}	31.3±6.7^{ab}	53.7±7.2^{bc}	68.0±2.9^{cd}	80.3±6.2^{cd}	86.3±12.9^{de}	76.5±6.8^{cd}	93.6±11.9^{de}	112.1±14.9^{e}	92.9±8.9^{de}	<0.0001
Sep15[3]	55.5±10.4	67.9±13.5	75.0±15.3	93.1±10.5	92.4±2.3	90.4±1.9	91.5±5.2	103.4±0.9	100.9±2.4	97.9±7.8	0.01
Txnrd3	48.6±8.6^{a}	59.3±3.0^{ab}	84.6±16.1^{c}	85.1±3.3^{c}	89.5±3.7^{cd}	101.3±10.2^{cd}	78.7±4.5^{bc}	100.1±2.3^{cd}	113.6±6.0^{d}	94.0±9.6^{cd}	0.0004
Kidney											
Gpx1	21.3±2.1^{a}	42.8±7.7^{ab}	65.6±4.4^{bc}	87.1±1.8^{cde}	85.7±10.2^{cde}	99.3±12.1^{de}	116.2±8.0^{e}	101.5±7.8^{de}	100.8±6.5^{de}	80.2±19.9^{cd}	<0.0001
Gpx3	40.1±4.7^{a}	53.6±21.3^{ab}	73.1±24.6^{ab}	86.5±5.3^{bc}	88.5±10.0^{bc}	94.7±11.1^{bc}	103.7±11.4^{c}	97.9±20.9^{bc}	107.6±10.4^{c}	89.4±6.0^{bc}	0.05
Selh	40.8±5.7^{a}	60.2±4.1^{b}	75.4±5.7^{bc}	87.5±1.0^{cd}	98.9±6.3^{d}	96.4±4.6^{d}	106.6±10.3^{d}	92.9±9.9^{cd}	97.2±4.5^{d}	99.0±4.2^{d}	<0.0001
Selk	59.5±2.2^{a}	71.3±3.3^{ab}	85.3±8.1^{bc}	88.9±5.7^{bcd}	92.8±2.4^{cd}	91.5±3.2^{cd}	104.0±4.4^{cd}	92.3±2.0^{cd}	91.3±4.7^{cd}	108.0±14.1^{d}	0.0008
Sepn1	56.1±6.3^{a}	70.6±7.2^{ab}	95.5±9.4^{ab}	71.4±8.2^{ab}	99.0±18.6^{ab}	88.7±11.2^{ab}	77.0±2.5^{ab}	97.3±12.2^{ab}	93.2±5.7^{ab}	103.1±5.2^{b}	0.04
Sepw1	29.0±1.6^{a}	51.1±6.1^{b}	74.4±3.8^{cd}	70.9±5.1^{c}	85.2±8.6^{cde}	88.8±8.5^{cde}	83.3±6.0^{cde}	113.6±1.8^{f}	92.9±4.7^{de}	96.9±6.4^{ef}	<0.0001
Sephs2	234.5±62.0^{b}	140.9±29.1^{a}	102.9±12.0^{a}	117.5±7.3^{a}	89.1±8.1^{a}	94.8±5.9^{a}	124.2±7.4^{a}	92.7±10.3^{a}	88.6±3.0^{a}	104.2±13.1^{a}	0.008
Txnrd1[3]	60.0±1.3^{a}	71.8±7.6^{ab}	82.2±2.9^{abc}	92.7±3.6^{abc}	86.6±0.8^{abc}	100.1±1.8^{bc}	118.3±0.7^{c}	94.8±8.9^{abc}	93.3±2.0^{abc}	96.0±12.2^{abc}	<0.0001
Muscle											
Gpx3	48.2±3.0^{a}	83.9±7.4^{b}	98.5±6.6^{bc}	98.1±6.3^{bc}	117.8±1.7^{c}	103.6±6.3^{bc}	87.5±15.6^{bc}	106.4±11.6^{bc}	94.5±16.8^{bc}	91.2±4.9^{bc}	0.005
Selh	53.9±8.3^{a}	47.1±9.5^{a}	93.6±13.0^{ab}	102.7±13.1^{b}	85.3±13.8^{ab}	91.1±14.6^{ab}	119.2±22.5^{b}	76.6±13.8^{ab}	83.8±13.0^{ab}	123.7±17.1^{b}	0.02
Sepw1	29.3±3.2^{a}	29.9±8.7^{a}	80.0±6.3^{ab}	100.7±10.4^{ab}	68.3±4.2^{ab}	98.2±21.8^{ab}	105.0±33.8^{b}	81.4±28.1^{ab}	84.4±37.2^{ab}	140.2±23.8^{b}	0.04

[1]Values are presented as mean±SEM, n = 3/diet. Means in a row with superscripts without a common letter differ significantly, $P < 0.05$.
[2]Relative selenoprotein mRNA values determined by qRT-PCR, determined in triplicate for each sample of total RNA isolated from the indicated tissue, and normalized to the average of Gapdh and Actb mRNA in the same sample, and expressed as a percentage of Se-adequate plateau levels.
[3]Differences determined by Scheffé's test as variances were not homogeneous.

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SUPPLEMENTAL TABLE 5 Selenium regulation of the selenoproteome in rat liver, kidney, and muscle[1]

Gene[2]	Name	Liver	Kidney	Muscle
Dio1	Deiodinase, iodothyronine, type I	Moderate	Low	Low
Dio2	Deiodinase, iodothyronine, type II	Low	Low	Low
Dio3	Deiodinase, iodothyronine, type III	Low	Low	Low
Gpx1	Glutathione peroxidase 1	**Very High**	High	High[3]
Gpx2	Glutathione peroxidase 2	Low	Low	Low
Gpx3	Glutathione peroxidase 3	High	High	Moderate
Gpx4	Glutathione peroxidase 4	Low	Low	Low
Selh	Selenoprotein H	High	High	Moderate
Seli	Selenoprotein I	Low	Low	Low
Selk	Selenoprotein K	High	Moderate	Low
Selm	Selenoprotein M	Low	Low	Low
Sepn1	Selenoprotein N	Moderate	Moderate	Low
Selo	Selenoprotein O	Low	Low	Low
Sepp1 (SelP)	Selenoprotein P	Moderate	Low	Low
Sepx1 (MsrB1)	Methionine-R-sulfoxide reductase	Low	Low	Low
Sels	Selenoprotein S	Low	Low	Low
Selt	Selenoprotein T	Moderate	Low	Low
Selv	Selenoprotein V	Not Present	Not Present	Low
Sepw1	Selenoprotein W	High	High	High
Sep15	Selenoprotein 15	Moderate	Low	Low
Sephs2 (SPS2)	Selenophosphate synthetase 2	Low	Moderate-Up[4]	Low
Txnrd1	Thioredoxin reductase 1	Low	Moderate	Low
Txnrd2	Thioredoxin reductase 2	Low	Low	Low
Txnrd3	Thioredoxin reductase 3	Moderate	Low	Low
Totals:	**Very High**	1	0	0
	High	4	4	1 (+1 NS)
	Moderate	6	4	2
	Low	12	15	20
	Not Present	1	1	0

[1]Susceptibility of all 24 selenoproteins in the rat selenoproteome to Se regulation in Se-deficient tissues. Selenoprotein mRNA levels were determined by qRT-PCR to compare levels in Se-deficient verus Se-adequate (0.24 µg Se/g diet) rats: **very high** = <10.9% of Se-adequate; high = 11-40.9% of Se-adequate; moderate = 41-70% of Se-adequate; Low = >70% of Se-adequate; not present = not detected by qRT-PCR.
[2]Gene symbol with common alternative symbol given in parentheses.
[3]Se-deficient muscle Gpx1 mRNA decreases to 37% of Se-adequate levels but with $P = 0.14$
[4] Kidney Sephs2 mRNA is moderately up-regulated in Se deficiency.