

Alleviation of lipid disorder and liver damage in high-fat diet-induced obese mice by selenium-enriched *Cardamine violifolia* with a high cadmium accumulation

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Table S1. Primer sequences for qPCR

Gene	Forward, 5' - 3'	Reverse, 5' - 3'
<i>Abca1</i>	CCAACATCTGAAAAACAGGTTTGGA	GACACTTCCCGGAAACGCAA
<i>Abcg1</i>	CTACATCTCCTATGTCAGATACGGC	ACTTCTGGAAGTGGCATGTCT
<i>Abcg5</i>	CCTGAACTTCACTTGTGGTGGA	TCTCGATGAACTGGACCCCT
<i>Abcg8</i>	CATCCTCGGAGACACGATGA	ACAGGAAGCCGTAGCTGATG
<i>Actb</i>	ACTGCCGCATCCTCTTCCT	GCCACAGGATTCCATACCCAA
<i>Atgl</i>	ACCATCTGCCTTCCAGACTGT	TAGGGCCTCACTGTAGGTGG
<i>Dgat1</i>	CATGATGGCTCAGGTCCCAC	ATACATGAGCACAGCCACCG
<i>Dgat2</i>	CATCACCACCGTCGTGGG	TCAAAGAGCTTCACCAGGGC
<i>Dio1</i>	CCTGTGGTGGTGGACACAAT	GGGCCAGCTTTACCCTTGTA
<i>Dio3</i>	AAGGGGTGGAAGAGGGACAT	TGCGAACCTCGCAGATTGAT
<i>Gpat1</i>	AAGGACATCGGGGTTTTCAA	GAGAGATCGCTACAGCACCA
<i>Gpat2</i>	TCAAAGATCTGGGGTACTTCA	TCCAGCTTGTCCTGATTGTCC
<i>Gpx1</i>	CTCTGAGGCACCACGATCC	TTGCCATTCTCCTGGTGTCC
<i>Gpx4</i>	TTGATAAGAACGGCTGCGTG	AGGTCCTTCTCTATCACCTGGG
<i>Hmgcr</i>	AAGAATCTTGTGGGCTCGGC	ACATTCTGTGCTGCATCCTGG
<i>Hsl</i>	CTGTGCACCTTGTGGCTTG	GCCATGCGGCAGATCTTCTA
<i>Ldlr</i>	CCTACCCCTCAAGACAGATGGT	AAGCTTGGCAATGGATCCCG
<i>Lipc</i>	GGAGAGACGCAGCAAAGAATG	AAGACTTTCTCCTGGCTCGG
<i>Lpl</i>	AAGAAGTCTGGCTGACACTGG	AACCCACTTTCAAACACCCAA
<i>Msrbl</i>	GGCCACGAGTTCCTGAATGA	GCAGTCCATGTCCTAGTGCC
<i>Mt1</i>	TCCTGCAAGAAGAGCTGCTG	GTTCGTACATCAGGCACAG
<i>Mt2</i>	TCCTGCAAGAAAAGCTGCTG	GCACTTGTCGGAAGCCTCTT
<i>Mtfl</i>	CAATGTTCCAGAATTCGACGA	CTGTCAATCAAGGCAGCTGTC
<i>Selenof</i>	ACAGATCAAGTATGTTGAGGC	CTCGCTCAGGAACTCTTCCA
<i>Selenoh</i>	AACAGCCGTGTTGAACTCTGGA	TGAGGACTCTTTCCCAACCTCT

Gene	Forward, 5' - 3'	Reverse, 5' - 3'
<i>Selenoi</i>	TCTCCTCTTGGTTGTGGCAG	GCTTCACCACTTGTACGCCA
<i>Selenok</i>	GCTGGTGGATGAGGAAGGTAAA	CTCTTCACCGCTTGATGGCT
<i>Selenom</i>	GTGATGAAGCACCTCCCTGG	GGGATTTCGCTCTAGTTCCTGG
<i>Selenoo</i>	GAGACTTTTCAGAGGTGCGG	TTGCTCCTCAGTGCTCCTTG
<i>Selenop</i>	TGGCCGTCTTGTGTATCACC	TCAAGACTCGTGAGATTGCAG
<i>Selenos</i>	CCTTTGCGAGGAGGTGGTTA	AGTTTCAGCCGCCAGATGAT
<i>Selenot</i>	GCACCGAAAACCTCTTTTACACT	CAACGAGCCTGCCAAGAAAC
<i>Selenow</i>	ACTGGTGACCGCCATCAAAG	GAGCTTTCAGGACCCTGCCT
<i>Sephs2</i>	GCCACCTGACTTGGTCTCTG	TTCAGCCTCTCCCAGTCCTT
<i>Sqle</i>	TGGGTTGCTTTCAATATTGTCTC	GGCGTAGATTGCAACGGAAA
<i>Txnrd1</i>	CCGGTCTGTGCAGAGATATTCA	GCTTAACCTCAGCAGCCAGA
<i>Txnrd2</i>	GGATCAAGTGTGGGGCTTCA	GCAACCAGTCACAGTAGGCT
<i>Txnrd3</i>	CCACCTGTGGTGAGGTATTCA	TGACAGGGAAACCCAACAGC

Abca1, ATP binding cassette subfamily A member 1; *Abcg1/ 5/ 8*, ATP binding cassette subfamily G member 1/ 5/ 8; *Actb*, actin beta; *Atgl*, adipose triglyceride lipase; *Dgat1/ 2*, diacylglycerol O-acyltransferase 1/ 2; *Dio1/ 3*, iodothyronine deiodinase 1/ 3; *Gpat1/ 2*, glycerol-3-phosphate acyltransferase 1/ 2; *Gpx1/ 4*, glutathione peroxidase 1/ 4; *Hmgcr*, 3-hydroxy-3-methylglutaryl coenzyme A reductase; *Hsl*, hormone-sensitive lipase; *Ldlr*, low density lipoprotein receptor; *Lipc*, lipase C hepatic type; *Lpl*, lipoprotein lipase; *MsrB1*, methionine sulfoxide reductase B1; *Mtf1*, metal regulatory transcription factor 1; *Mt1/ 2*, metallothionein 1/ 2; *Selenof/ h/ i/ k/ m/ o/ p/ s/ t/ w*, selenoprotein F/ H/ I/ K/ M/ O/ P/ S/ T/ W; *Sephs2*, selenophosphate synthetase 2; *Sqle*, squalene epoxidase; *Txnrd1/ 2/ 3*, thioredoxin reductase 1/ 2/ 3.

Table S2. Target protein, type, dilution, and source of primary antibodies used for the Western blot analyses.

Target protein	Source	Isotype	Dilution	Brand (Cat. #)
Actb	Mouse	IgM	1:5,000	Proteintech (60008-1-Ig)
Dio1	Rabbit	IgG	1:2,000	Proteintech (11790-1-AP)
Dio3	Rabbit	IgG	1:2,000	Bioss (Bs-3902R-A488)
Gpx1	Rabbit	IgG	1:1,000	Abcam (ab22604)
Gpx4	Rabbit	IgG	1:5,000	Abcam (ab125066)
Mt	Rabbit	IgG	1:1,000	Abnova (MAB9787)
Mtf1	Rabbit	IgG	1:1,000	Boster (A04733-3)
Selenof	Rabbit	IgG	1:5,000	Abcam (ab124840)
Selenoh	Rabbit	IgG	1:1,500	Abcam (ab151023)
Selenoi	Rabbit	IgG	1:1,000	Abcam (ab157571)
Selenoo	Rabbit	IgG	1:5,000	Abcam (ab172957)
Selenop	Rabbit	IgG	1:1,000	Abcam (ab109514)
Selenos	Rabbit	IgG	1:1,000	Proteintech (15591-1-AP)
Sod1	Rabbit	IgG	1:200	Santa Cruz Biotechnology (sc-11407)
Txnrd1	Rabbit	IgG	1:3,000	Proteintech (11117-1-AP)
Txnrd2	Rabbit	IgG	1:3,000	Proteintech (16360-1-AP)

Actb, actin beta; Dio1/ 3, iodothyronine deiodinase 1/ 3; Gpx1/ 4, glutathione peroxidase 1/ 4; Mt, metallothionein; Mtf1, metal regulatory transcription factor 1; Selenof/ h/ i/ o/ p/ s, Selenoprotein F/ H/ I/ O/ P/ S; Sod1, superoxide dismutase 1; Txnrd1 /2, thioredoxin reductase 1/ 2.

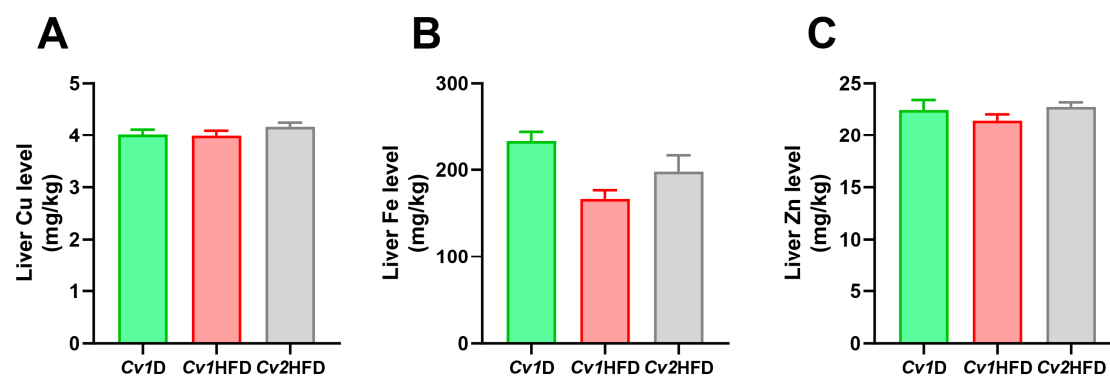


Figure S1. Accumulation of copper (Cu) (A), iron (Fe) (B), and zinc (Zn) (C) in the liver of adult female mice. Data are means \pm SEM ($n = 10 - 12$) without differences, $P > 0.05$. *Cv1D*, control diet with 0.15 mg Se and 0.03 mg Cd (in the form of control *Cv*)/kg; *Cv*, *Cardamine violifolia*; *Cv1HFD*, high-fat diet with 0.15 mg Se and 0.03 mg Cd (in the form of control *Cv*)/kg; *Cv2HFD*, high-fat diet with 1.5 mg Se and 0.29 mg Cd (in the form of Se-enriched *Cv*)/kg.

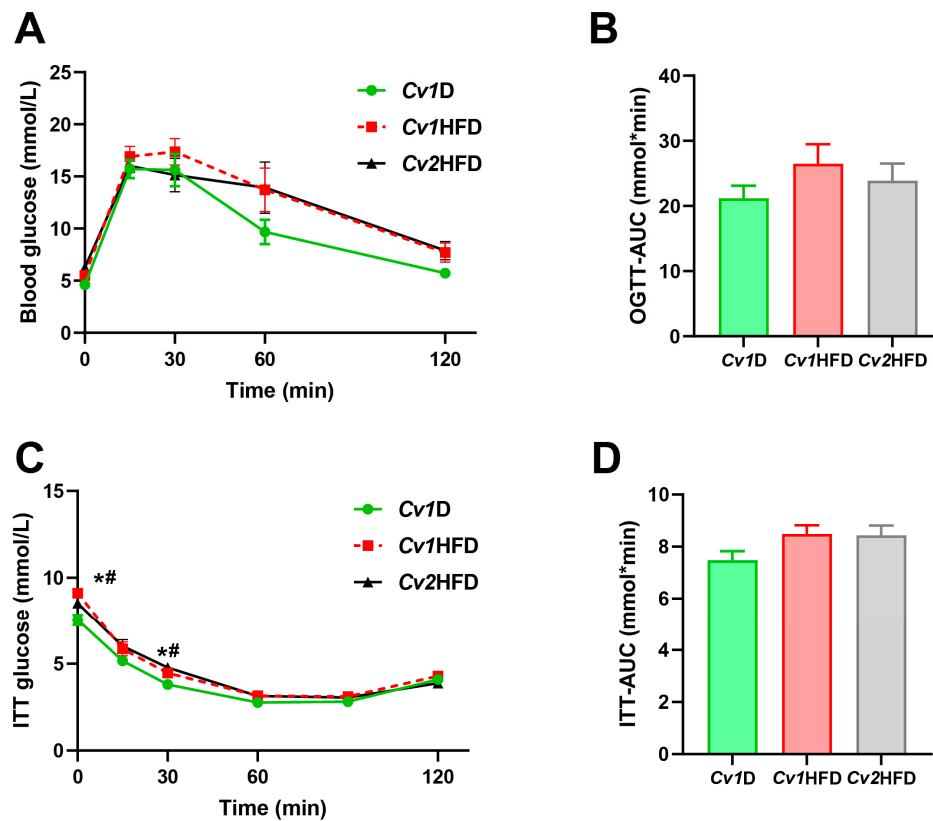


Figure S2. Blood glucose changes in oral glucose tolerance test (OGTT) (A - B) and insulin tolerance test (ITT) (C - D) on adult female mice. Data are means \pm SEM ($n = 10$) and differ with * between the Cv/D and Cv/HFD groups or # between the Cv/D and Cv2HFD groups, $P < 0.05$. AUC, area under curve; Cv/D, control diet with 0.15 mg Se and 0.03 mg Cd (in the form of control Cv)/kg; Cv, *Cardamine violifolia*; Cv/HFD, high-fat diet with 0.15 mg Se and 0.03 mg Cd (in the form of control Cv)/kg; Cv2HFD, high-fat diet with 1.5 mg Se and 0.29 mg Cd (in the form of Se-enriched Cv)/kg; ITT, insulin tolerance test; OGTT, oral glucose tolerance test.

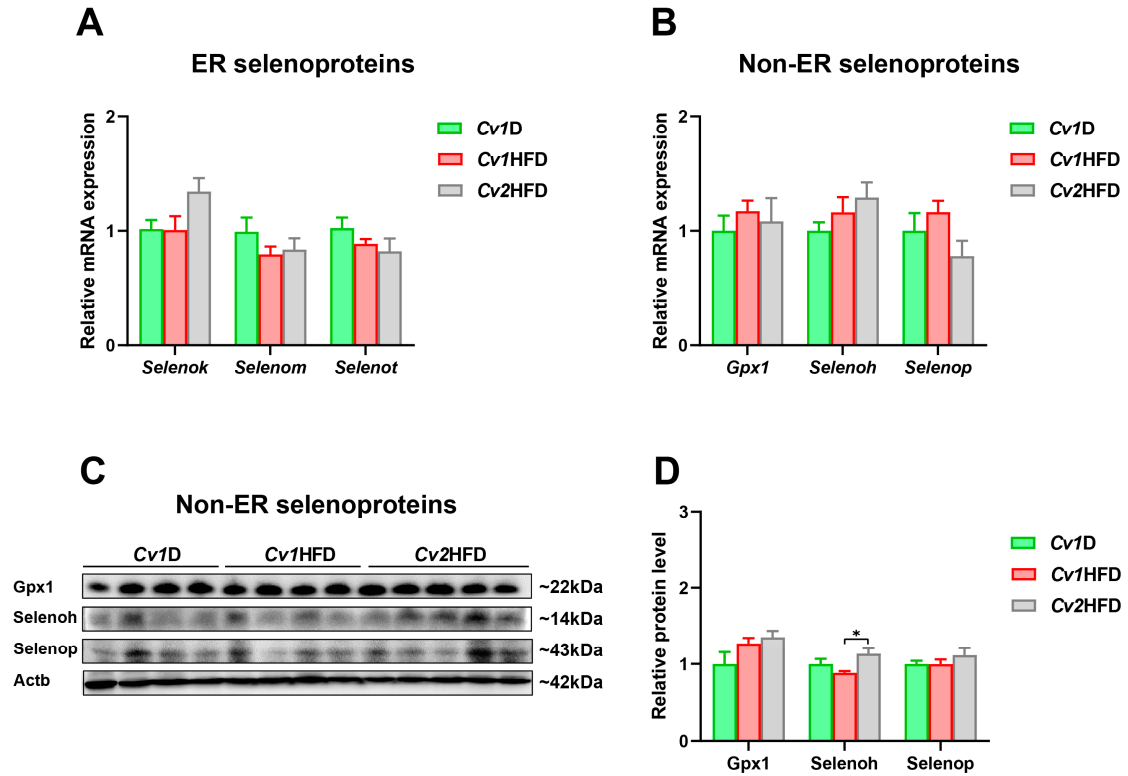


Figure S3. The relative mRNA expression of three endoplasmic reticulum (ER) selenoproteins (A) and three non-ER selenoproteins. (B) in the liver of female mice. (C) A representative immunoblot image for Gpx1, Selenoh and Selenop. (D) The relative protein levels of Gpx1, Selenoh and Selenop. Data are means \pm SEM ($n = 8 - 10$) and differ with *, $P < 0.05$. Cv1D, control diet with 0.15 mg Se and 0.03 mg Cd (in the form of control Cv)/kg; Cv, *Cardamine violifolia*; Gpx1, glutathione peroxidase 1; Cv1HFD, high-fat diet with 0.15 mg Se and 0.03 mg Cd (in the form of control Cv)/kg; Cv2HFD, high-fat diet with 1.5 mg Se and 0.29 mg Cd (in the form of Se-enriched Cv)/kg; Selenoh /k /m /p /t, selenoprotein h /k /m /p /t.