

High Dietary Selenium Intake Alters Lipid Metabolism and Protein Synthesis in Liver and Muscle of Pigs^{1–3}

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Abstract

Background: Prolonged high intakes of dietary selenium have been shown to induce gestational diabetes in rats and hyperinsulinemia in pigs.

Objective: Two experiments were conducted to explore metabolic and molecular mechanisms for the diabetogenic potential of high dietary selenium intakes in pigs.

Methods: In Expt. 1, 16 Yorkshire-Landrace-Hampshire crossbred pigs (3 wk old, body weight = 7.5 ± 0.81 kg, 50% males and 50% females) were fed a corn-soybean meal basal diet supplemented with 0.3 or 1.0 mg Se/kg (as selenium-enriched yeast for 6 wk). In Expt. 2, 12 pigs of the same crossbreed (6 wk old, body weight = 16.0 ± 1.8 kg) were fed a similar basal diet supplemented with 0.3 or 3.0 mg Se/kg for 11 wk. Biochemical and gene and protein expression profiles of lipid and protein metabolism and selenoproteins in plasma, liver, muscle, and adipose tissues were analyzed.

Results: In Expt. 1, the 1-mg-Se/kg diet did not affect body weight or plasma concentrations of glucose and nonesterified fatty acids. In Expt. 2, the 3-mg-Se/kg diet, compared with the 0.3-mg-Se/kg diet, increased ($P < 0.05$) concentrations of plasma insulin (0.2 compared with 0.4 ng/mL), liver and adipose lipids (41% to 2.4-fold), and liver and muscle protein (10–14%). In liver, the 3-mg-Se/kg diet upregulated ($P < 0.05$) the expression, activity, or both of key factors related to gluconeogenesis [phosphoenolpyruvate carboxykinase (PEPCK); 13%], lipogenesis [sterol regulatory element binding protein 1 (*SREBP1*), acetyl-coenzyme A carboxylase (ACC), and fatty acid synthase (FASN); 46–90%], protein synthesis [insulin receptor (INSR), P70 ribosomal protein S6 kinase (P70), and phosphorylated ribosomal protein S6 (P-S6); 88–105%], energy metabolism [AMP-activated protein kinase (AMPK); up to 2.8-fold], and selenoprotein glutathione peroxidase 3 (GPX3; 1.4-fold) and suppressed ($P < 0.05$) mRNA levels of lipolysis gene cytochrome P450, family 7, subfamily A, polypeptide 1 (*CYP7A1*; 88%) and selenoprotein gene selenoprotein W1 (*SEPW1*; 46%). In muscle, the 3-mg-Se/kg diet exerted no effect on the lipid profiles but enhanced ($P < 0.05$) expression of P-S6 and mammalian target of rapamycin (mTOR; 42–176%; protein synthesis); selenoprotein P (SELP; 40-fold); and tumor suppressor protein 53 (P53) and peroxisome proliferator-activated receptor γ (*PPARG*; 52–58%; lipogenesis) and suppressed ($P < 0.05$) expression of INSR (59%; insulin signaling); selenoprotein S (*SELS*); deiodinases, iodothyronine, type I (*DIO1*); and thioredoxin reductase 1 (*TXNRD1*; 50%; selenoproteins); and *ACC1* and FASN (35–51%; lipogenesis).

Conclusion: Our research showed novel roles, to our best knowledge, and mechanisms of high selenium intakes in regulating the metabolism of protein, along with that of lipid, in a tissue-specific fashion in pigs. *J Nutr* 2016;146:1625–33.

Keywords: AMPK, lipid, protein, Se, selenoprotein

Introduction

Our laboratory previously showed that the overproduction of glutathione peroxidase (GPX)⁴ 1, a selenoprotein, induced type 2 diabetes-like phenotypes in mice at the age of 6 mo (1). Although dietary selenium restriction in mice ameliorated most of the phenotypes (2), prolonged feeding of a high-selenium diet (3 mg Se/kg) caused mild gestational diabetes in pregnant rats and insulin resistance in their offspring (3). Likewise, 2 pig studies (4, 5) showed the potential of high selenium intakes in causing hyperinsulinemia.

Furthermore, several large human studies showed an alarming association between a high selenium intake and/or body selenium status and increased risk of hyperglycemia, hyperlipidemia, or type 2 diabetes (6–8). However, the molecular mechanism for the diabetogenic potential of a high selenium intake remains unclear.

Dietary selenium and/or GPX1 production (9) affected the functional expression of important proteins involved in gluconeogenesis [phosphoenolpyruvate carboxykinase (PEPCK)] and

glycolysis [glucokinase (GK)] (10), insulin signaling [insulin receptor (INSR), insulin receptor substrate 1 (IRS1), and v-akt murine thymoma viral oncogene homolog 2 (AKT2)], and lipid metabolism (11) [sterol regulatory element binding protein (SREBP) 1 and SREBP2, acetyl-CoA carboxylase 1 (ACC1); cholesterol 7 α -hydroxylase (CYP7A1), PPARG, fatty acid synthase (FASN), and tumor suppressor p53 (P53)] (12–17). Alterations of these factors were associated with expressions of 12 selenoproteins (1, 3, 5, 9, 18, 19). However, to the best of our knowledge, there was no information on effects of dietary selenium, in particular high selenium intake, on body protein metabolism, although protein synthesis was reported to negatively regulate insulin sensitivity (20, 21) and a high protein diet inhibited the development of type 2 diabetes (22). Mammalian target of rapamycin (mTOR), ribosomal protein S6 kinase (P70), ribosomal protein S6 (S6), factor 4E binding protein 1 (4EBP1), and eukaryotic translation initiation factor 4E (EIF4E) are key factors involved in the protein synthesis pathway (23–27). Furthermore, AMP-activated protein kinase (AMPK) plays a key role in regulating energy metabolism (28) and showed a negative association with selenium intake in rats (29). The expression or function of AMPK seems to be affected by superoxide (30, 31) or a high-fat diet (32). However, the role of AMPK in the high-selenium-intake-induced phenotype is unknown.

Pigs represent an excellent model to study human nutrition and medicine because of their great similarities in digestive systems (33), nutrient metabolism, and responses to high selenium intakes (4, 5). Furthermore, pigs are an important food-producing species worldwide, and selenium supplementation is required in diets for pigs. Therefore, 2 pig experiments were conducted, with the use of a control diet of 0.3 mg Se/kg, to determine the following: 1) whether a moderately high selenium intake (1.0 mg Se/kg diet) produced diabetogenic or hyperlipidemic effects similar to those of a 3.0-mg-Se/kg diet; 2) whether the 3.0-mg-Se/kg diet was able to affect protein metabolism and amino acid and FA profiles in liver, muscle, and adipose tissue; and 3) how the 3.0-mg-Se/kg diet exerted its metabolic effects in those tissues.

Methods

Animals, diets, and management. Our animal protocols were approved by the Institutional Animal Care and Use Committee of Cornell University. All pigs used in both experiments were weanling crossbreeds (Yorkshire-Landrace-Hampshire) selected from the Cornell

University Swine Farm, with half males and half females. Expt. 1 was conducted to determine if a moderately high dietary selenium intake affected plasma glucose and lipid concentrations. A total of 16 weanling pigs (3 wk old, body weight = 7.5 ± 0.81 kg) were divided into 2 groups ($n = 8/\text{group}$) and fed a corn-soybean meal basal diet (BD₁; Supplemental Table 1) supplemented with 0.3 or 1.0 mg Se/kg (as selenium-enriched yeast; courtesy of ADM Alliance Nutrition) for 6 wk. Expt. 2 was conducted, on the basis of the results of Expt. 1, to determine if and how a high-selenium diet (3.0 mg Se/kg) altered lipid and protein metabolism and the underlying mechanism in liver, muscle, and adipose tissue of pigs. A total of 12 pigs (6 wk old, body weight = 16 ± 1.8 kg) were fed a similar corn-soybean meal basal diet (BD₂; Supplemental Table 1) supplemented with 0.3 or 3.0 mg Se/kg (selenium supplemented as selenium-enriched yeast ($n = 6/\text{group}$)) for 11 wk. Pigs in both experiments were allotted to treatment groups on the basis of body weight, litter, and sex and were housed in pens with a concrete floor in a temperature-controlled barn (22–25°C) with a light-dark cycle of 12:12 h. Pigs were given free access to feed and water, and orts and feed intakes were recorded daily. Individual body weights of pigs were recorded biweekly.

Plasma biochemical analyses. Whole-blood samples of individual pigs were collected at week 0 and then biweekly after overnight food deprivation (8 h) to prepare plasma samples as previously described (3). Plasma concentrations of total TGs, total cholesterol (TC), nonesterified fatty acids (NEFAs), glucose, and insulin (at weeks 6 and 11) were analyzed as previously described (9).

Liver, muscle, and adipose biochemical analyses. At the end of Expt. 2, all pigs were killed after overnight (8 h) food deprivation (5) to collect samples of liver, muscle (longissimus dorsi), and adipose tissue (retroperitoneal fat) (5). The samples were snap-frozen in liquid nitrogen and stored at -80°C until analyses. Total lipids were extracted from liver (50 mg) and adipose tissue (50 mg) for analyses of TG, TC, and NEFA concentrations (34). Total FAs were extracted from the liver (250 mg) and adipose tissue (50 mg) (35) for the subsequent analyses by using a GC system (HP 6890; Hewlett Packard). Protein concentrations in the liver and muscle were determined following a previously described method (36). Total amino acids were extracted from the liver (500 mg) and muscle (500 mg) (37), and the subsequent analyses of amino acid profiles were performed on an HPLC system (Nexera X2; Shimadzu).

Real-time qPCR, immunoblotting, and enzyme activity analyses. Total mRNA was isolated from liver (20 mg) and muscle (20 mg), and the subsequent quantification was carried out by real-time qPCR (7900 HT; Applied Biosystems) of key genes involved in insulin signaling and lipid metabolism and selected selenoprotein genes as previously described (9). The primer sequences used for all of the assayed genes are shown in Supplemental Table 2. The $2^{-\Delta\Delta\text{Ct}}$ (ddCt) method was used for the quantification with β -actin (ACTB) as a reference gene, and the relative abundance was normalized to the control (as “1”). The validity of ACTB as the reference was verified by its amplification plots and dissociation curves of all samples from the liver and muscle samples (Supplemental Figure 1). GPX, GK, and PEPCK activities were measured in the liver and/or muscle (38), and FASN activity was measured in the liver and muscle (39, 40). Western blot analyses of key factors in the pathways of protein synthesis, insulin signaling, energy metabolism, and selected selenoproteins were performed as previously described (9); and the relative density of the protein bands was quantified by using ImageJ software (NIH) and normalized to ACTB as the loading control. The relative density of the phosphorylated protein bands was quantified by using ImageJ software and normalized first to ACTB as the loading control and then to the relative density of their corresponding total protein bands. The primary antibodies used for the Western blot analyses are presented in Supplemental Table 3.

Statistical analyses. Data were analyzed by using the general linear model procedure of SAS (SAS Institute). The main effects of dietary treatments on body weight and plasma biochemical measures were subjected to 1-factor ANOVA with time-repeated measurements (3).

¹ Supported in part by NIH DK 53018.

² Author disclosures: Z Zhao, M Barcus, J Kim, KL Lum, C Mills, and XG Lei, no conflicts of interest.

³ Supplemental Tables 1–5 and Supplemental Figures 1–3 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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⁴ Abbreviations used: ACC, acetyl-CoA carboxylase; ACTB, β -actin; AKT2, v-akt murine thymoma viral oncogene homolog 2; AMPK, AMP-activated protein kinase; CYP7A1, cytochrome P450, family 7, subfamily A, polypeptide 1; DIO1, deiodinases, iodothyronine, type I; DIO3, deiodinases, iodothyronine, type III; EIF4E, eukaryotic initiation factor 4E; FASN, fatty acid synthase; GK, glucokinase; GPX, glutathione peroxidase; INSR, insulin receptor; IRS1, insulin receptor substrate 1; mTOR, mammalian target of rapamycin; NEFA, nonesterified fatty acid; PEPCK, phosphoenolpyruvate carboxykinase; PPARG, peroxisome proliferator-activated receptor γ ; P-S6, phosphorylated ribosomal protein S6; P53, tumor suppressor protein 53; P70, P70 ribosomal protein S6 kinase; S6, ribosomal protein S6; SELH, selenoprotein H; SELS, selenoprotein S; SELT, selenoprotein T; SEPN1, selenoprotein N; SEPP1 (SELP), selenoprotein P; SEPW1, selenoprotein W; SREBP, sterol regulatory element binding protein; TC, total cholesterol; TXNRD1, thioredoxin reductase 1.

TABLE 1 Body weight and plasma glucose and lipid concentrations of pigs fed 0.3 or 1.0 mg Se/kg for 6 wk (Expt. 1)¹

	Diet		SE, mg Se/kg	<i>P</i> (time × Se)
	0.3 mg Se/kg	1.0 mg Se/kg		
Body weight, kg				0.7
0 wk	7.23 ^d	7.24	0.43	
2 wk	14.3 ^c	14.3	0.91	
4 wk	22.8 ^b	23.3	1.74	
6 wk	34.8 ^a	35.3	2.02	
Plasma glucose, mg/dL				0.1
0 wk	134 ^a	189	16.8	
2 wk	101 ^b	106	5.64	
4 wk	87 ^c	93	3.3	
6 wk	89 ^c	90	3.2	
Plasma cholesterol, mg/dL				0.3
0 wk	84.5 ^a	79.8	3.49	
2 wk	59.5 ^c	42.2 [*]	4.17	
4 wk	65.3 ^{bc}	64.4	3.55	
6 wk	70.1 ^b	70.2	3.62	
Plasma TGs, mg/dL				0.9
0 wk	58.2 ^a	46.3	5.41	
2 wk	58.7 ^a	42.2 [*]	4.11	
4 wk	40.6 ^b	31.2 [*]	1.68	
6 wk	42.8 ^b	31.1 [*]	2.13	
Plasma nonesterified fatty acids, μmol/L				0.8
0 wk	137 ^d	175	23.0	
2 wk	459 ^c	413	44.4	
4 wk	533 ^b	512	74.6	
6 wk	655 ^a	766	110	

¹ Values are means, *n* = 6. Within a column, labeled means without a common superscript letter differ, *P* < 0.05. *Different from 0.3 mg Se/kg at that time, *P* < 0.05.

Data generated from qPCR, Western blot, and biochemical assays of tissue samples were analyzed by using SAS with Student's *t* test. For all of the analyses, pooled SEs were listed and the significance level for differences was *P* < 0.05.

Results

Expt. 1. The 1-mg-Se/kg diet exerted no significant effect on body weight or plasma concentrations of glucose and NEFAs at any of the time points. However, pigs fed 1.0 mg Se/kg had 23–28% lower (*P* < 0.05) plasma TG concentrations than did those fed 0.3 mg Se/kg. Pigs fed the high-selenium diet also had lower (*P* < 0.05) plasma TC concentrations than those fed the control diet at week 2 (Table 1).

Expt. 2. Pigs fed the high-selenium diet (3 mg Se/kg) had lower (*P* < 0.05) body weight at weeks 4 and 8 than pigs fed 0.3 mg Se/kg, but the difference between the 2 groups at week 11 was not significant (*P* = 0.098; Table 2). Thus, there was no difference (*P* = 0.30) in the overall average daily body weight gain (0.76 ± 0.042 compared with 0.76 ± 0.039 kg), average daily feed intake (1.9 ± 0.13 compared with 1.6 ± 0.12 kg), or gain-to-feed ratio (0.44 ± 0.021 compared with 0.47 ± 0.024) between the 2 groups. The high-selenium-fed pigs had a 1-fold higher (*P* < 0.05) plasma insulin concentration at the end of experiment (week 11) than pigs fed 0.3 mg Se/kg. Although pigs fed the high-selenium diet showed 23% lower (*P* < 0.05) plasma

glucose concentrations and higher (*P* < 0.05) plasma NEFA concentrations than those fed 0.3 mg Se/kg at week 2, the high selenium supplementation produced no effect on either of the measures at other time points (midinterval data not shown) or on plasma TGs or TC at any time point.

In contrast, TC and TG concentrations in the adipose tissue of pigs fed the high-selenium diet were 2.4-fold and 41% greater (*P* < 0.05), respectively, than those fed 0.3 mg Se/kg (Figure 1A, B), whereas NEFA concentrations in adipose tissue were unaffected by the high-selenium diet (Supplemental Figure 2A). Likewise, hepatic concentrations of TC, TGs, and NEFAs in pigs fed the high-selenium diet were 40%, 2.3-fold, and 63% greater (*P* < 0.05), respectively, than those in pigs fed 0.3 mg Se/kg (Figure 1C–E). However, the high-selenium supplementation exerted no effect on these measures of lipid profiles in muscle tissue (Supplemental Figure 2B–D). Compared with those fed 0.3 mg Se/kg, the high-selenium-fed pigs showed greater concentrations (*P* < 0.05) of myristic acid, palmitic acid, stearic acid, behenic acid, oleic acid, linoleic acid, and palmitoleic acid and lower concentrations (*P* < 0.05) of dihomono-γ-linolenic acid in liver and lower concentrations (*P* < 0.05) of stearic acid, oleic acid, α-linolenic acid, eicosatrienoic acid, and behenic acid in adipose tissue (Table 3, Supplemental Table 4). Total protein concentrations in the liver (10.1 ± 0.253 compared with 11.5 ± 0.253 g/g dry tissue) and muscle (12.3 ± 0.121 compared with 13.6 ± 0.237 g/g dry tissue) were 14% and 10% greater (*P* < 0.05), respectively, in pigs fed the high-selenium diet than those fed 0.3 mg Se/kg. The high-selenium diet increased (*P* < 0.05) concentrations of aspartic acid, glutamine, and alanine in muscle but not in liver (Supplemental Table 5).

TABLE 2 Body weight and plasma biochemical indicators of pigs fed 0.3 or 3.0 mg Se/kg for 11 wk (Expt. 2)¹

	Diet		SE, mg Se/kg	<i>P</i> (time × Se)
	0.3 mg Se/kg	3.0 mg Se/kg		
Body weight, kg				0.1
0 wk	16.0 ^d	15.8	0.518	
4 wk	42.4 ^c	38.3 [*]	1.03	
8 wk	68.9 ^b	61.7 [*]	1.62	
11 wk	82.7 ^a	78.5	2.05	
Plasma glucose, mg/dL				0.003
2 wk	123 ^a	95 [*]	4.97	
11 wk	121 ^b	119	2.04	
Plasma cholesterol, mg/dL				0.2
2 wk	77.0 ^b	78.5	2.18	
11 wk	81.1 ^a	81.5	2.01	
Plasma TGs, mg/dL				0.7
2 wk	46.1 ^a	38.5	3.00	
11 wk	25.8 ^b	27.8	2.49	
Plasma nonesterified fatty acids, μmol/L				0.1
2 wk	287 ^a	619 [*]	56.6	
11 wk	195 ^b	245	34.2	
Plasma insulin, ng/mL				0.2
6 wk	0.3	0.2	0.04	
11 wk	0.2	0.4 [*]	0.03	

¹ Values are means, *n* = 6. Within a column, labeled means without a common superscript letter differ, *P* < 0.05. *Different from 0.3 mg Se/kg at that time, *P* < 0.05. Only the data of the final time points, those with the diet effect and initial body weight of pigs, are shown in the table.

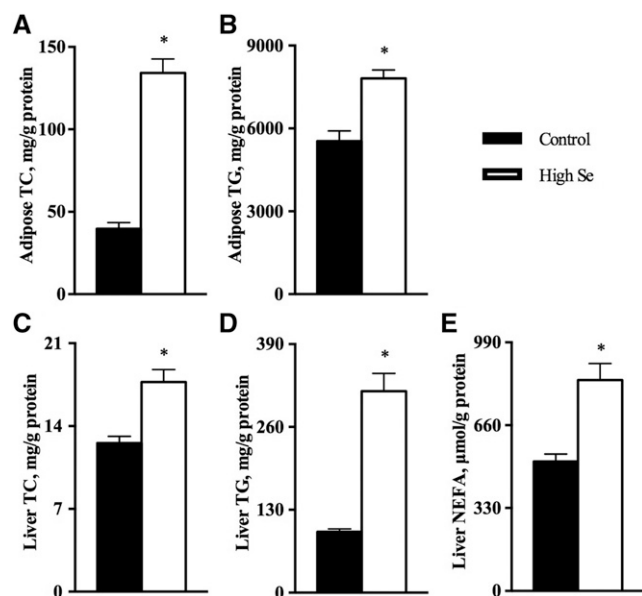


FIGURE 1 Adipose tissue concentrations of TC (A) and TGs (B) and liver concentrations of TC (C), TGs (D), and NEFAs (E) in pigs fed 0.3 or 3.0 mg Se/kg for 11 wk (Expt. 2). Values are means \pm SEs, $n = 6$. *Different from control, $P < 0.05$. Control = 0.3 mg Se/kg; high Se = 3.0 mg Se/kg. NEFA, nonesterified fatty acid; TC, total cholesterol.

Compared with the 0.3-mg-Se/kg diet, the high-selenium diet enhanced ($P < 0.05$) mRNA levels of *GPX3* in both the liver and muscle but decreased ($P < 0.05$) mRNA levels of selenoprotein W (*SEPW1*) in the liver and selenoprotein S (*SELS*), deiodinases, iodothyronine, type 1 (*DIO1*), and thioredoxin reductase 1 (*TXNRD1*) in the muscle (Figure 2A, B). The mRNA levels of the other 7 selenoprotein genes [*GPX1*, *GPX4*, selenoprotein P (*SEPP1*), selenoprotein H (*SEPH*), selenoprotein N (*SEPN1*), selenoprotein T (*SELT*), and deiodinases, iodothyronine, type 3 (*DIO3*)] were not affected by the high-selenium diet. Compared with those fed the 0.3-mg-Se/kg diet, pigs fed the high-selenium diet showed 59% greater ($P < 0.05$) *SREBP1* and 88% lower ($P < 0.01$) *CYP7A1* mRNA levels in the liver (Figure 2C) as well as 42–48% greater ($P < 0.05$) peroxisome proliferator-activated receptor γ (*PPARG*) and *P53* and 51% lower ($P < 0.05$) *ACC1* mRNA levels in the muscle (Figure 2D). The high-selenium diet doubled ($P < 0.05$) the mRNA levels of *FASN* in the liver but resulted in a 57% decrease ($P = 0.12$) in the muscle compared with the 0.3-mg-Se/kg diet. The high-selenium diet showed no effect on mRNA levels of *INSR*, *IRS1*, *AKT2*, *GK*, or *SREBP2* in either liver or muscle.

The high-selenium diet elevated ($P < 0.05$) *GPX3* (1.4-fold) and *INSR* (88%) protein amounts but showed no effect on selenoprotein P (*SELP*) protein in the liver (Figure 3A, Supplemental Figure 3A). Meanwhile, the high-selenium diet resulted in 41-fold higher ($P < 0.01$) *SELP*, 59% lower ($P < 0.05$) *INSR*, and no change in *GPX3* protein in the muscle (Figure 3B, Supplemental Figure 3B). Whereas total *AKT* protein in either liver or muscle was affected by the high-selenium diet (Supplemental Figure 3A, B), hepatic amounts of phosphor-*AMPK* α to total *AMPK* α protein were 2.8-fold greater ($P < 0.05$), whereas hepatic amounts of phosphor-*AMPK* β to total *AMPK* β were 38% lower ($P < 0.05$) in the high-selenium-fed pigs than in controls (Figure 3A). The high-selenium diet also enhanced ($P < 0.05$) hepatic phosphor-*ACC* to total *ACC* (23%) (Figure 3A) but decreased ($P < 0.05$) muscle phosphor-*ACC* to total *ACC*

(31%) (Figure 3B). The high-selenium diet enhanced ($P < 0.05$) phosphor-S6 to S6 and total P70 in the liver (1-fold) (Figure 3A) and phosphor-S6 to S6 and mTOR protein in the muscle (42% to 1.8-fold) (Figure 3B).

The high-selenium diet upregulated ($P < 0.05$) *GPX* activity in the liver and muscle by 21% and 57%, respectively (Figure 4A, B). The high-selenium diet increased ($P < 0.05$) hepatic *FASN* activity by 46% but decreased the enzyme activity ($P < 0.05$) in muscle by 34% (Figure 4C, D). The high-selenium diet increased ($P < 0.05$) hepatic *PEPCK* activity by 13% (Figure 4E). There was no significant effect of the high-selenium diet on activities of *GK* in liver (133 ± 13.2 compared with 162 ± 8.19 U/g protein) or of both *GK* (14 ± 1.9 compared with 14 ± 1.1 U/g protein) and *PEPCK* (14 ± 0.95 compared with 13 ± 0.87 U/g protein) in muscle.

Discussion

The present study showed 3 unique metabolic responses in growing pigs to high dietary selenium intakes. First, although plasma lipid profiles were not affected by either the 1- or 3-mg-Se/kg diet, the 3-mg-Se/kg (high-selenium) diet elevated TGs, TC, and NEFAs in liver and/or adipose tissue up to 3-fold compared with control pigs. In contrast, there was no such effect of the high-selenium diet on muscle lipid profiles. Second, the high-selenium diet enhanced concentrations of 3 SFAs and 5 unsaturated FAs in the liver but decreased concentrations of 5 FAs, including 3 unsaturated FAs in muscle. Third, the high-selenium diet elevated total protein concentrations in both liver and muscle and concentrations of aspartic acid, glutamine, and alanine in muscle. It appears that the high-selenium diet altered lipid and protein metabolism in a tissue-specific fashion. The lack of a consistent effect of the high-selenium diets on plasma lipid profiles indicates that measuring these changes only in plasma without examining internal organs may not be a reliable method to assess the true metabolic effects of high dietary selenium intakes (41). However, several human studies showed a positive association between serum/plasma selenium concentrations and lipid concentrations (42–44). The high-selenium diet

TABLE 3 FA profiles in liver and adipose tissue of pigs fed 0.3 or 3.0 mg Se/kg for 11 wk (Expt. 2)¹

	Diet		SE, mg
	0.3 mg Se/kg	3.0 mg Se/kg	Se/kg
Liver, mg/g tissue			
Myristic acid (14:0)	0.1 ^b	0.2 ^a	0.01
Palmitic acid (16:0)	4.5 ^b	6.9 ^a	0.67
Palmitoleic acid (16:1)	0.1 ^b	0.3 ^a	0.02
Stearic acid (18:0)	6.9 ^b	9.3 ^a	0.40
Oleic acid (18:1n-9c)	3.6 ^b	5.4 ^a	0.29
Linoleic acid (18:2n-6c)	3.9 ^b	4.9 ^a	0.13
Dihomo-γ-linolenic acid (20:3n-6)	0.2 ^a	0.0 ^b	0.01
Behenic acid (22:0)	3.7 ^b	4.7 ^a	0.21
Adipose tissue, mg/g tissue			
Stearic acid (18:0)	29 ^a	21 ^b	1.9
Oleic acid (18:1n-9c)	56 ^a	42 ^b	3.1
α-Linolenic acid (18:3n-3)	1.0 ^a	0.7 ^b	0.049
Eicosatrienoic acid (20:3n-3)	0.6 ^a	0.0 ^b	0.012
Behenic acid (22:0)	0.2 ^a	0.0 ^b	0.011

¹ Values are means, $n = 6$. Means in a row without a common superscript letter differ, $P < 0.05$.

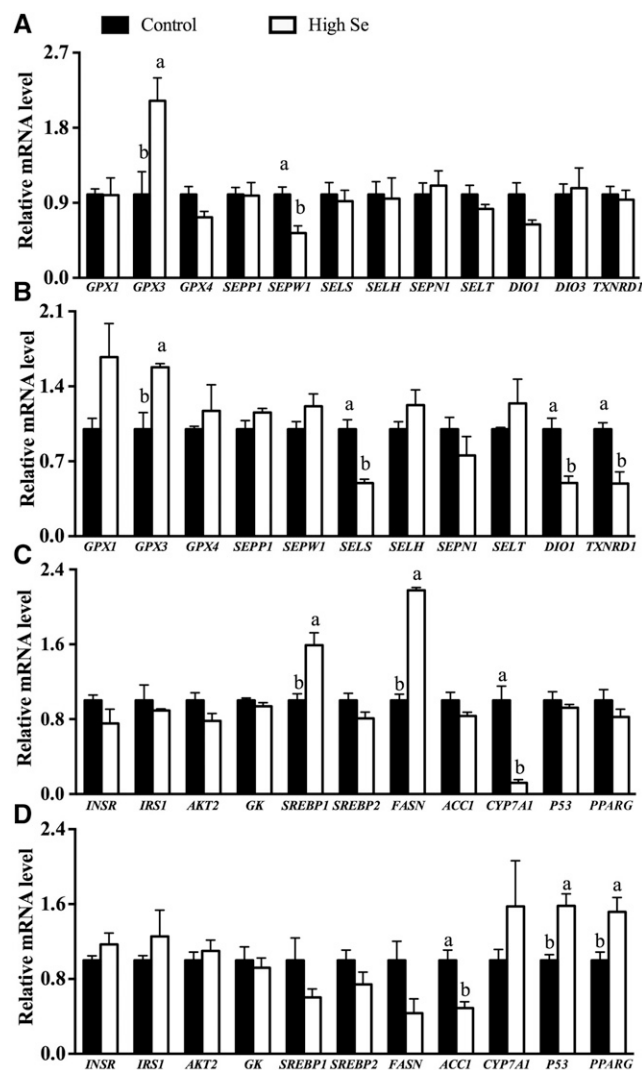


FIGURE 2 Relative mRNA levels of selenoproteins in the liver (A) and muscle (B) and molecules of insulin signaling, lipid synthesis, and lipid hydrolysis pathways in the liver (C) and muscle (D) of pigs fed 0.3 or 3.0 mg Se/kg for 11 wk (Expt. 2). Values are means \pm SEs, $n = 6$. Means without a common letter differ, $P < 0.05$. Control = 0.3 mg Se/kg; high Se = 3.0 mg Se/kg. ACC1, acetyl-CoA carboxylase 1; AKT2, v-akt murine thymoma viral oncogene homolog 2; CYP7A1, cytochrome P450, family 7, subfamily A, polypeptide 1; DIO1, deiodinases, iodothyronine, type I; DIO3, deiodinases, iodothyronine, type III; FASN, fatty acid synthase; GK, glucokinase; GPX, glutathione peroxidase; INSR, insulin receptor; IRS1, insulin receptor substrate 1; P53, tumor suppressor protein 53; PPARG, peroxisome proliferator-activated receptor γ ; SELH, selenoprotein H; SELS, selenoprotein S; SELT, selenoprotein T; SEPN1, selenoprotein N; SEPP1, selenoprotein P; SEPW1, selenoprotein W; SREBP, sterol regulatory element binding protein; TXNRD1, thioredoxin reductase 1.

did not elevate fasting plasma concentrations of glucose at any time point but increased insulin at week 11 in Expt. 2. Although we measured plasma insulin at only 2 time points (weeks 6 and 11) and were unable to perform insulin tolerance tests in the growing pigs, the high-selenium-intake-induced hyperinsulinemia appeared only at the late stage of the feeding, which was consistent with the findings from 2 previous studies (4, 5) and a downregulation of INSR in the muscle. Although pigs fed the high-selenium diet had lower body weight than those fed the control diet at weeks 4 and 8, the difference was not significant

($P = 0.10$) at week 11 in Expt. 2. The lack of a striking overt phenotype in response to the high-selenium intake might reflect a progressive adaption of the body to the metabolic effect of selenium supranutrition, and our observed biochemical changes were early events instead of endpoints. Therefore, a longer feeding period may be needed to define the full effect of a high-selenium intake on body nutrient metabolism, including activity in pigs.

The high-selenium diet-induced elevations of hepatic TG, TC, and NEFA concentrations were associated with upregulation of gene expression of *SREBP1* and *FASN* (9), protein production of total ACC, and activities of FASN and PEPCK, along with a downregulation of *CYP7A1* mRNA. Because the liver is a main organ of lipogenesis in pigs, an elevated functional expression of *SREBP1* and *FASN* could stimulate the pathway (45). The enhanced PEPCK activity, as the rate-limiting step of

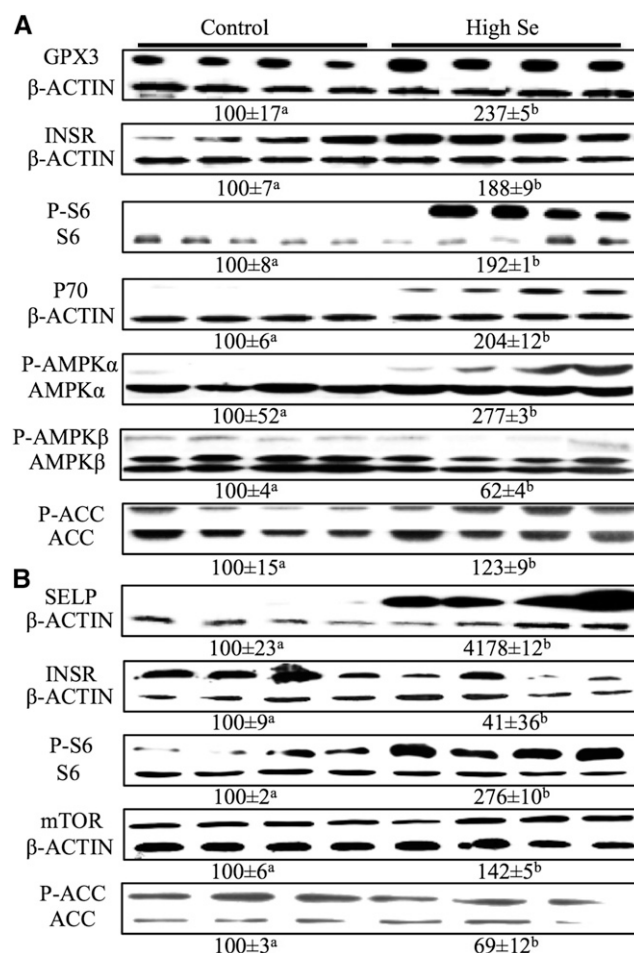


FIGURE 3 Relative protein concentrations of liver GPX3, INSR, P-S6, P70, P-AMPK α , AMPK α , P-AMPK β , P-ACC, and ACC (A) and muscle SELP, INSR, P-S6, P-ACC, and mTOR (B) in pigs fed 0.3 or 3.0 mg Se/kg for 11 wk (Expt. 2). Values below the protein band were relative densities and are expressed as means \pm SEs, $n = 4-6$. Means without a common letter differ, $P < 0.05$. Control = 0.3 mg Se/kg; high Se = 3.0 mg Se/kg. ACC, acetyl-CoA carboxylase; AMPK α , AMP-activated protein kinase α ; AMPK β , AMP-activated protein kinase β ; GPX3, glutathione peroxidase 3; INSR, insulin receptor; mTOR, mammalian target of rapamycin; P70, P70 ribosomal protein S6 kinase; P-ACC, phosphorylated acetyl-CoA carboxylase; P-AMPK α , phosphorylated AMP-activated protein kinase α ; P-AMPK β , phosphorylated AMP-activated protein kinase β ; P-S6, phosphorylated ribosomal protein S6; SELP, selenoprotein P; S6, ribosomal protein S6.

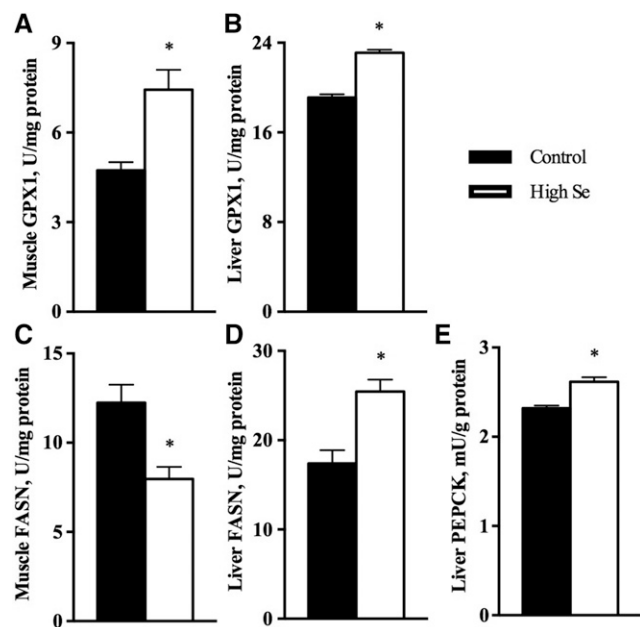


FIGURE 4 GPX activities in muscle (A) and liver (B), FASN activities in muscle (C) and liver (D), and PEPCK activities in liver (E) of pigs fed 0.3 or 3.0 mg Se/kg for 11 wk (Expt. 2). The GPX activity unit is defined as 1 μ mol NAD(P)H used/min. The FASN activity unit is defined as 1 mmol NAD(P)H used/min. Values are means \pm SEs, $n = 6$. *Different from control, $P < 0.05$. Control = 0.3 mg Se/kg; high Se = 3.0 mg Se/kg. FASN, fatty acid synthase; GPX, glutathione peroxidase; PEPCK, phosphoenolpyruvate carboxykinase.

gluconeogenesis (46, 47), might help generate glucose as the carbon source for FA synthesis. The increased total ACC should also support lipogenesis, although the increased phosphor-ACC might inhibit ACC complex activity (48). Because the CYP7A1

enzyme is involved in the cholesterol hydrolysis pathway (49), the decrease of 90% in its mRNA level by the high-selenium diet might contribute to the accumulation of hepatic TC. It is intriguing to find effects of a high-selenium diet on gene expression or on the activities of FASN and ACC in the muscle that are opposite to those in the liver because the muscle lipid profile was not affected by the high-selenium diet. Although adipose tissue showed lipid accumulation similar to that in the liver in response to the high-selenium diet, we did not determine whether that accumulation was a direct effect of the high-selenium diet in the adipose tissue or a secondary response to the accumulation in the liver (as the main organ for storing lipids) (50).

Likewise, the opposite effects of the high-selenium diet on FA profiles between the liver (increase) and adipose tissue (decrease) remain intriguing. Notably, 4 SFAs [14:0 (myristic acid), 16:0 (palmitic acid), 18:0 (stearic acid), and 22:0 (behenic acid)] and 2 MUFAs [16:1 (palmitoleic acid), 18:1n-7c (oleic acid)] were elevated by the high selenium intake. A previous report showed a positive correlation between SFAs and obesity (51), whereas MUFAs could reduce TGs with high glucose and low fat intakes (52). However, previous research has suggested that long-chain MUFAs actually contributed to the development of obesity (53). Seemingly, the SFA elongation pathway in the liver of pigs was upregulated by the high selenium intake. In contrast, 2 PUFAs (18:3n-3 and 20:3n-3) in adipose tissue were greatly suppressed by the high selenium intake. Because PUFAs may help induce FA β -oxidation (54), the decreased PUFAs in the adipose tissue, combined with the increased SFAs and MUFAs in the liver, in pigs fed the high-selenium diet might also suppress lipolysis, contributing to the lipid accumulation in these tissues.

The high-selenium diet led to increases of 10–14% in concentrations of liver and muscle proteins, which was associated with stimulated signaling for protein synthesis of mTOR, P70, and phosphor-S6 (55). Although it is unclear to us how 3 specific

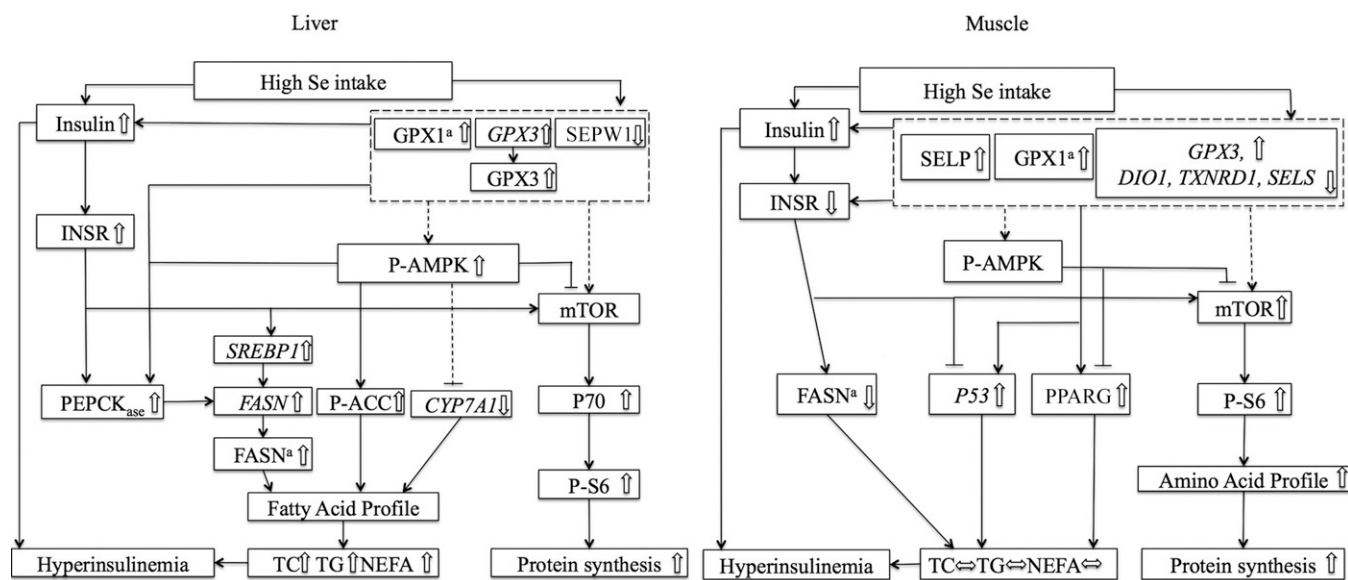


FIGURE 5 Scheme of postulated regulatory pathways and mechanisms for the effects of feeding a high-selenium diet on lipid and protein metabolism in tissues of pigs. The solid lines represent data-supported pathways, whereas the dashed lines indicate potential, unapproved pathways. *CYP7A1*, cytochrome P450, family 7, subfamily A, polypeptide 1; *DIO1*, deiodinases, iodothyronine, type I; *FASN*, fatty acid synthase; *GPX*, glutathione peroxidase; *INSR*, insulin receptor; *mTOR*, mammalian target of rapamycin; *NEFA*, nonesterified fatty acid; *P53*, tumor suppressor protein 53; *P70*, P70 ribosomal protein S6 kinase; *P-ACC*, phosphorylated acetyl-CoA carboxylase; *P-AMPK*, phosphorylated AMP-activated protein kinase; *P-S6*, phosphorylated ribosomal protein S6; *PEPCK_{ase}*, phosphoenolpyruvate carboxykinase activity; *PPARG*, peroxisome proliferator-activated receptor γ ; *SELP*, selenoprotein P; *SELS*, selenoprotein S; *SEPW1*, selenoprotein W; *SREBP1*, sterol regulatory element binding protein 1; *TC*, total cholesterol; *TXNRD1*, thioredoxin reductase 1.

amino acids were elevated in the muscle by the high selenium intake, our results showed a novel function of dietary selenium in regulating metabolism of not only glucose (56) and FAs (9) but also of amino acids. This finding implies a common role of dietary selenium in the overall fuel nutrient metabolism and is reinforced by the high-selenium diet-induced increases in AMPK α and phosphor-AMPK α (activation of the kinase) and decreases in phosphor-AMPK β (inactivation of the kinase) in the liver (48). Because AMPK is a key upstream regulator of PEPCK, SREBP1, p53, ACC, PPAR α , CYP7A1, and mTOR (57–59), the stimulation of this enzyme could help explain the accelerated lipogenesis and protein synthesis in the liver. However, AMPK was not affected by the high-selenium diet in muscle. It is also difficult to understand the mechanism and implication of high dietary selenium intake in increasing hepatic INSR but decreasing the protein in the muscle. Because muscle represents the largest insulin-responsive tissue in the body (60), downregulation of INSR and upregulation of the mTOR-S6 pathway (49) might impair insulin signaling, contributing to the hyperinsulinemia that is an early event of insulin resistance. Similar to the responses of several FFAs in the liver and adipose tissue to the high selenium intake, the physiologic relevance or molecular mechanism for the rather minor elevations of 3 amino acids in the muscle by the 3-mg-Se/kg diet remains unclear.

Our fundamental interest in the present study was to elucidate if the metabolic effects of the high dietary selenium intake were ultimately mediated by specific selenoproteins. As shown in our previous studies (3, 18), the high-selenium diet resulted in a moderate (20–50%) increase in GPX activities in both liver and muscle. Overproduction of GPX activity by *Gpx1* overexpression in mice led to type 2 diabetes-like phenotypes, including hyperlipidemia and fatty liver (1, 9, 19), and the selenium deficiency partially rescued those disorders (9). The high-selenium diet induced gene expression of *GPX3* in both liver and muscle and elevated the GPX3 protein in the liver. Although it is unclear why the high-selenium diet induced the gene expression but not the protein production of GPX3 in the muscle, an increase in GPX3 has been shown to be involved in the development of type 2 diabetes (61). Another very striking tissue-specific response was the 41-fold increase in SELP in the muscle, but no change in the liver, with the high-selenium diet. Because previous human and animal studies have suggested that SELP acts as a “hepatokine” and a negative regulator of insulin signaling (62–65), its substantial increase in the muscle might also contribute to the development of insulin resistance and hyperinsulinemia. In addition, the high-selenium diet down-regulated the gene expression of *SEPW1* in the liver and of *SELS*, *DIO1*, and *TRXND1* in muscle. In one of our previous pig studies (5), gene expressions of 8 selenoproteins in 6 tissues were decreased by both dietary selenium deficiency and excess (3 mg Se/kg). The regulatory mechanism and the physiologic implications for these types of paradoxical responses remain a question for further research.

In summary, our study shows a new role for selenium in regulating protein and lipid metabolism through the activation of AMPK and the differential responses of selenoproteins between different tissues. As shown in Figure 5, the induced hepatic lipid accumulation by a high selenium intake was associated with stimulation of lipogenesis and gluconeogenesis and the suppression of lipolysis, along with an increase in 4 SFAs and 2 MUFAs in the liver and a decrease in 2 PUFAs in adipose tissue. The high-selenium diet-induced slight increases in liver and muscle protein concentrations were associated with an elevated protein synthesis pathway and 3 amino acids in the muscle. The unique activation

of hepatic AMPK reinforced the role of a high selenium intake in regulating overall nutrient metabolism. The metabolic differential effects of a high dietary selenium intake in different tissues might be mediated by specific selenoproteins. The stimulated hepatic GPX3 might be involved in regulating hepatic lipid deposition and the substantially elevated SELP in muscle might contribute to the development of insulin resistance and hyperinsulinemia.

Acknowledgments

XGL designed the research and had primary responsibility for the final content; ZZ, MB, JK, KLL, CM, and XGL conducted the experiments and analyzed the data; and ZZ, KLL, and XGL wrote the manuscript. All authors read and approved the final manuscript.

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