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# Targeted Metabolomics Analysis Reveals that Dietary Supranutritional Selenium Regulates Sugar and Acylcarnitine Metabolism Homeostasis in Pig Liver

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## ABSTRACT

**Background:** The association between high selenium (Se) intake and metabolic disorders such as type 2 diabetes has raised great concern, but the underlying mechanism remains unclear.

**Objective:** Through targeted metabolomics analysis, we examined the liver sugar and acylcarnitine metabolism responses to supranutritional selenomethionine (SeMet) supplementation in pigs.

**Methods:** Thirty-six castrated male pigs (Duroc-Landrace-Yorkshire, 62.0 ± 3.3 kg) were fed SeMet adequate (Se-A, 0.25 mg Se/kg) or SeMet supranutritional (Se-S, 2.5 mg Se/kg) diets for 60 d. The Se concentration, biochemical, gene expression, enzyme activity, and energy-targeted metabolite profiles were analyzed.

**Results:** The Se-S group had greater fasting serum concentrations of glucose (1.9-fold), insulin (1.4-fold), and free fatty acids (FFAs,1.3-fold) relative to the Se-A group ($P < 0.05$). The liver total Se concentration was 4.2-fold that of the Se-A group in the Se-S group ($P < 0.05$), but expression of most selenoprotein genes and selenoenzyme activity did not differ between the 2 groups. Seven of 27 targeted sugar metabolites and 4 of 21 acylcarnitine metabolites significantly changed in response to high SeMet ($P < 0.05$). High SeMet supplementation significantly upregulated phosphoenolpyruvate carboxy kinase (PEPCK) activity by 64.4% and decreased hexokinase and succinate dehydrogenase (SDH) activity by 46.5–56.7% ($P < 0.05$). The relative contents of glucose, dihydroxyacetone phosphate, $\alpha$-ketoglutarate, fumarate, malate, erythrose-4-phosphate, and sedoheptulose-7-phosphate in the Se-S group were 21.1–360% greater than those in the Se-A group ($P < 0.05$). The expression of fatty acid synthase (FASN) and the relative contents of carnitine, hexanoyl-carnitine, decanoyl-carnitine, and tetradecanoyl-carnitine in the Se-S group were 35–97% higher than those in the Se-A group ($P < 0.05$).

**Conclusions:** Dietary high SeMet-induced hyperglycemia and hyperinsulinemia were associated with suppression of sugar metabolism and elevation of lipid synthesis in pig livers. Our research provides novel insights into high SeMet intake-induced type 2 diabetes. *J Nutr* 2020;00:1–8.

**Keywords:** supranutritional supplementation, SeMet, sugar and acylcarnitine metabolism, liver, pig

## Introduction

Selenium (Se) is an essential trace element for health in humans and other animal species, and plays a critical role in redox regulation, immunity, and thyroid hormone metabolism (1). These biological functions of Se are mainly mediated by 24–25 mammalian selenoproteins (2). In humans, dietary Se deficiency has been associated with poor immunity and an increased risk of mortality and cancer (3). Supranutritional Se intake has been prevalent worldwide (especially in America and European Union countries), not only to prevent Se deficiency, but also because of Se's known antioxidant effects and potential to aid in the prevention of cancer and cardiovascular diseases (4, 5). However, there is a U-shaped association between Se intake or status and the risk of metabolic disorders in humans (6), and supranutritional supplementation may induce adverse health effects in the population with adequate Se (7). Specifically, the link between type 2 diabetes and populations with high Se intake has caused great concern.

The Nutritional Prevention of Cancer trial has demonstrated that long-term supplementation with Se (200 $\mu$g/d),


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as compared with placebo treatment, significantly increases the risk of type 2 diabetes (8). Similarly, several human studies have revealed that high plasma Se status is associated with elevated fasting plasma glucose and the prevalence of type 2 diabetes (9–12). Furthermore, experimental animal model studies in rodents have demonstrated that supranutritional Se intake potentiates hyperglycemia, hyperinsulinemia, and type 2 diabetes (13–15). However, the mechanism through which high Se intake induces potentially diabetogenic changes in humans remains incompletely understood.

Glutathione peroxidase (*GPX*) and thioredoxin reductase (*TXNRD*) are the most well-studied selenoprotein families with antioxidant function (16). Previous studies have shown that overexpression or knockout of the *GPX1* gene in mice alters redox homeostasis and glucose and lipid metabolism; thus, resulting in type 2 diabetes-like phenotypes (17, 18). Likewise, studies in chickens have suggested that supranutritional Se administration alters blood glucose and insulin status, as well as the pancreatic redox state, through affecting the expression of various selenoprotein genes in the liver, muscle, and pancreas (19, 20). Moreover, research in pigs and mice has indicated that supranutritional dietary Se intake induces alterations in lipid metabolism, protein synthesis, and molecular targets related to energy metabolism in insulin target tissues (21–23). Together, these results indicate that high Se intake significantly affects glucose and lipid metabolism in various animals. Glycolysis, the tricarboxylic acid cycle, the pentose phosphate pathway, and acylcarnitine metabolism play important roles in controlling glucose homeostasis and lipid metabolism, but the roles of these metabolic processes in the type 2 diabetes-like phenotype induced by high Se intake remain unclear.

In addition, the toxicity, bioavailability, and metabolic functions of Se in animals depend in part on its chemical form (24–27). To our knowledge, there is little information about the effects of high Se supplementation as selenomethionine (SeMet) on the risk of type 2 diabetes in animal studies. Pigs are an excellent animal model for studies of human nutrition and medicine because their digestive and metabolic systems are highly similar to those in humans (28). Therefore, the present study was conducted to determine whether high SeMet intake (2.5 mg Se/kg diet) might cause phenotypes of type 2 diabetes in pigs, and to examine the responses of liver selenoprotein gene expression, redox state, and sugar and acylcarnitine metabolism to high SeMet supplementation.

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Supplemental Tables 1–5 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.
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Abbreviations used: ACSS2, acyl-CoA synthetase short chain family member 2; FASN, fatty acid synthase; GPX, glutathione peroxidase; IS, internal standard; LDH, lactic dehydrogenase; MDH, malate dehydrogenase; PEPCK, phosphoenolpyruvate carboxy kinase; SDH, succinate dehydrogenase; Se-A, selenium adequate; SeMet, selenomethionine; Se-S, selenium supranutritional; SELENOW, selenoprotein W; SREBF, sterol regulatory element binding transcription factor; TC, total cholesterol; TXNRD, thioredoxin reductase.

# Methods

## Animals, treatments, and sample collection

All animal procedures performed in this study were approved by the Animal Care and Use Committee of the Institute of Animal Sciences of Chinese Academy of Agricultural Sciences. A total of 36 castrated male finishing pigs (4-mo-old, Duroc-Landrace-Yorkshire) with a mean body weight 62.0 ± 3.3 kg were randomly divided into 2 groups with 6 replicates of 3 per cage. Before treatment, the pigs were fed a diet based on corn and soybeans (0.25 mg Se/kg diet). During the experiment, the animals were fed a similar basal diet (**Supplemental Table 1**, 0.1 mg Se/kg) supplemented to a final concentration of 0.25 mg Se/kg (Se adequate, Se-A) or 2.5 mg Se/kg (Se supranutritional, Se-S) provided as SeMet (98% purity, J&K Chemical) for 60 d. The concentrations of Se in the above diets were measured by inductively coupled plasma mass spectrometry. All pigs were given ad libitum access to feed and water. Blood samples were collected from the precaval vein after a fasting period of 12 h at the beginning of the experiment and after 30 and 60 d of experimentation. Blood was centrifuged at 1482 × *g* (4°C) for 10 min for serum separation and stored at −80°C. At the end of the experiment, 6 pigs that had been subjected to each treatment and fasted for 12 h were killed through electric shock and exsanguinated. The liver, semitendinous muscle, heart, and kidney were quickly collected, frozen with liquid nitrogen, and stored at −80°C.

## Determination of Se concentration, serum biochemistry, and liver enzyme activity

Se concentrations were determined with Agilent 7900 inductively coupled plasma mass spectrometry (Agilent Technologies), according to our previous report (29). Pig liver (GBW10051) and chicken muscle (GBW10018) supplied by the National Standard Substances Center were used as reference material to control for the accuracy of the analytical method. The amounts of Se in the reference material (GBW10051 and GBW10018) analyzed herein were consistent with the certificate concentrations (data not shown). Serum FFAs, total cholesterol (TC), TG, HDL cholesterol, LDL cholesterol, glucose, and insulin were determined with commercial kits provided by Zhongsheng Beikong Biotechnology, according to the manufacturer's instructions. Liver GPX, TXNRD, malondialdehyde, hexokinase, phosphoenolpyruvate carboxy kinase (PEPCK), lactic dehydrogenase (LDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), and fatty acid synthase (FASN) activity were analyzed with commercial kits provided by Nanjing Jiancheng Bioengineering Institute, according to the manufacturer's protocols.

## Selenoprotein mRNA expression

Total RNA was isolated from liver samples with an RNAprep pure tissue kit (Tiangen Biotech Co. Ltd), and the RNA purity and integrity were evaluated through agarose gel (1.2%) electrophoresis and the $OD_{260}/OD_{280}$ value. cDNA was synthesized from 2 $\mu$g of total RNA with a PrimeScript RT reagent Kit (TaKaRa), according to the manufacturer's protocol. The expression of selected mRNAs was detected with an Applied Biosystems QuantStudio™ Real-Time PCR system (ThermoFisher Scientific), with SYBR® Premix Ex Taq™ reagents (TaKaRa). The primers for selenoproteins and a housekeeping gene (*GAPDH*) were as described previously (13), and the primers for genes associated with sugar and lipid metabolism are shown in **Supplemental Table 2**. The relative mRNA abundance of selected genes was normalized to the expression of *GAPDH*, and then calculated with the $2^{-\Delta\Delta Ct}$ method.

## Targeted metabolomics analysis

Determination of sugar metabolites: 40 mg liver homogenate was mixed with 1.6 mL methanol/water (8/2) and centrifuged at 14,000 × *g* for 15 min at 4°C; then the supernatant was evaporated to dryness. The residues were reconstituted in 100 $\mu$L of 50% aqueous acetonitrile, containing 500 ng/mL of isotopic internal standards (IS) (Santa Cruz Biotechnology). The sample extracts were injected onto a BEH Amide column (100 mm × 2.1 mm, 1.7 $\mu$m) with a linear gradient elution

system mobile-phase of (A) 10 mM ammonium acetate and 0.1% ammonium hydroxide in water, and (B) 10 mM ammonium acetate and 0.1% ammonium hydroxide in acetonitrile/water (9/1) at 0.3 mL/min. The gradient elution conditions were 2 min, 100% B; 8 min, 0% B; 13 min, 0% B; 13.5 min, 100% B; and 17 min, 100% B. The ultra-performance LC system was coupled to a Triple Quad 5500 tandem mass spectrometer (AB Sciex) with negative electrospray ionization (30, 31). The target parent ions, 1 product ions, and retention times are shown in **Supplemental Table 3**. AB Sciex Analyst software (version 1.6.3) was used to control instrument acquisition and analyze the data. The relative amount of target metabolites was normalized to the peak area of the IS.

Quantification of acylcarnitine: 20 mg liver homogenate was mixed with 750 $\mu$L acetonitrile/isopropanol (3/1) and 250 $\mu$L deionized water, and centrifuged at 14,000 × *g* for 15 min at 4°C; then the supernatant was evaporated to dryness. The residue was redissolved in 200 $\mu$L of 50% aqueous methanol containing internal standards (Cambridge Isotope Laboratories), and then injected into a Phenomenex Luna Omega Polar C18 column (100 mm × 2.1 mm, 1.6 $\mu$m) at a flow rate of 0.4 mL/min. The mobile phase consisted of (A) water and (B) methanol both containing 0.1% formic acid. The chromatographic separation was conducted with a gradient elution program as follows: starting at 1% B and held for 1 min; 1.5 min, 60% B; 7 min, 95% B; 7.3 min, 100% B and held for 8.3 min; 8.4 min, and 1% B and held for 10 min. The mass spectrometer was operated in electrospray ionization mode according to previously published conditions (32, 33). The target parent ions, 1 product ions, and retention times are shown in **Supplemental Table 4**. Analyst software (AB Sciex, version 1.6.3) was used to control the instruments and acquire data. The relative content of acylcarnitines was normalized to the peak area of the corresponding IS.

### Statistical analysis

The data in this study are presented as means ± SEMs. Statistical analysis was performed in SPSS 22.0 software (SPSS, Inc.). For most variables, the 2 groups were compared with use of a 2-sided Student's *t* test (with Welch's correction for unequal variance). Serum Se data were analyzed by 1-factor ANOVA with time-repeated measurements. If there was a main effect or the interaction was significant, Duncan's multiple range test was used for post hoc comparisons of all 6 means. For all analyses, the significance level was set at $P < 0.05$. All figures were drawn in GraphPad Prism 7.0 software (GraphPad Software Inc.).

## Results

### Effects of supranutritional Se treatment on growth performance and Se concentrations in pigs

Supranutritional Se supplementation (2.5 mg/kg) did not significantly affect the pigs' daily weight gain, daily intake, and feed conversion ratio, as compared with that in the Se-A group (0.25 mg/kg) (**Supplemental Table 5**). At the start of the experiment, the serum Se concentrations were not significantly different between the 2 groups ($P > 0.05$); thus suggesting that the pigs in the 2 groups had comparable Se status. The serum Se concentrations in the Se-S group were 2.7 times (at 30 d) and 3.1 times (at 60 d) that of the Se-A group ($P < 0.001$) (**Table 1**). Similarly, the concentrations of Se in the liver, muscle, heart, and kidney tissues were 4.2, 6.7, 5.4, and 2.0 times that of the Se-A group in the Se-S group ($P < 0.001$), respectively (**Figure 1**A–D).

### Effects of supranutritional Se supplementation on serum biochemistry

After 60 d of treatment, the 2 groups did not differ in serum TC, TG, HDL cholesterol, or LDL cholesterol concentrations (Supplemental Table 5). Pigs fed the Se-S diet showed significantly higher ($P < 0.05$) fasting serum glucose concentrations than pigs in the Se-A group at the end of the experiment (**Figure 2**A). Correspondingly, the serum insulin and FFA concentrations in the Se-S group were 1.4 and 1.3 times that of the Se-A group ($P < 0.05$), respectively (Figure 2B and C).

**TABLE 1** Serum Se concentrations in pigs fed an Se-A (0.25 mg/kg) or Se-S (2.5 mg/kg) diet for 60 d[1]

| | Diet | | |
|---|---|---|---|
| | Se-A (0.25 mg Se/kg) | Se-S (2.5 mg Se/kg) | *P* (time × diet) |
| Serum Se, mg/L | | | <0.001 |
| 0 d | 0.14 ± 0.017 | 0.16 ± 0.016[c] | |
| 30 d | 0.17 ± 0.027 | 0.47 ± 0.064[b]* | |
| 60 d | 0.19 ± 0.011 | 0.59 ± 0.026[a]* | |

[1]Values are means ± SEMs, $n = 6$. Within a column, labeled means without a common superscript letter differ, $P < 0.05$. Se-A, selenium adequate; Se-S, selenium supranutritional.
*Different from Se-A at that time point, $P < 0.05$.

### Effects of supranutritional Se status on liver mRNA levels

The relative abundance of transcripts associated with selenoprotein, sugar, and lipid metabolism in the liver of pigs is shown in **Figure 3**. As compared with the levels in the Se-A group, expression of 3 of 13 selenoprotein genes [*GPX4*, *TXNRD1*, and selenoprotein W (*SELENOW*)] was significantly affected by supranutritional Se supplementation ($P < 0.05$). The abundance of *GPX4* in the Se-S group was 14.8% greater than that in the Se-A group ($P < 0.05$). The relative mRNA levels of *TXNRD1* and *SELENOW* in the Se-S group were 11.9% ($P < 0.05$) and 25.2% ($P < 0.05$) lower, respectively, compared with the Se-A group. As shown in Supplemental Table 5, there were no significant differences ($P > 0.05$) in the GPX and TXNRD activity, and the amount of malondialdehyde in the livers of

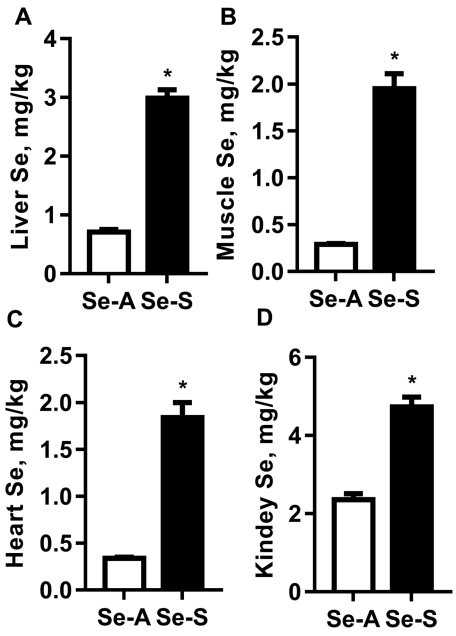


**FIGURE 1** Concentrations of Se in the liver (A), muscle (B), heart (C), and kidney (D) of pigs fed an Se-A (0.25 mg/kg) or Se-S (2.5 mg/kg) diet for 60 d. Values are means ± SEMs, $n = 6$. *Different from Se-A, $P < 0.05$. Se-A, selenium adequate; Se-S, selenium supranutritional.

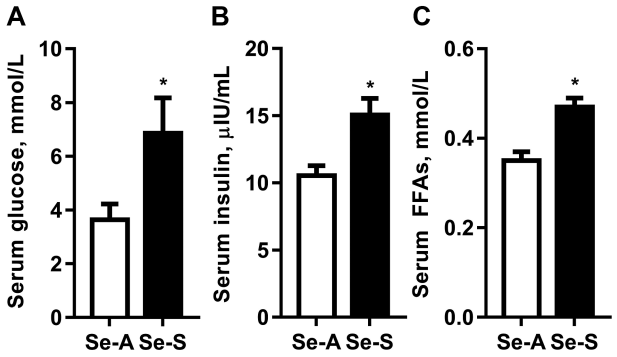


**FIGURE 2** Serum concentrations of glucose (A), insulin (B), and FFAs (C) in pigs fed an Se-A (0.25 mg/kg) or Se-S (2.5 mg/kg) diet for 60 d. Values are means ± SEMs, $n = 6$. *Different from Se-A, $P < 0.05$. Se-A, selenium adequate; Se-S, selenium supranutritional.

pigs in the 2 groups. In the Se-S group, the transcript abundance of *FASN*, sterol regulatory element binding transcription factor (*SREBF*) 2, and acyl-CoA synthetase short chain family member 2 (*ACSS2*) in the Se-S group was 19.2–34.6% greater than that in the Se-A group ($P < 0.05$). The amounts of cytochrome P450 family 7 subfamily A polypeptide-1 in the Se-S group were 46.6% lower ($P < 0.05$) than those in the Se-A group (Figure 3B).

### Effect of supranutritional Se supplementation on liver sugar and acylcarnitine metabolism and enzyme activity

To evaluate the response of liver sugar metabolism to supranutritional Se supplementation, we detected 27 metabolites associated with glycolysis, the tricarboxylic acid cycle, and the pentose phosphate pathway. The relative content of these metabolites is shown in Supplemental Table 3. The relative contents of glucose, dihydroxyacetone phosphate, α-ketoglutarate, fumarate, malate, erythrose-4-phosphate, and sedoheptulose-7-phosphate in the Se-S group were 52.9%, 3.6 times, 33.5%, 25.9%, 21.1%, 3.5 times, and 40.9% greater than those in the Se-A group ($P < 0.05$), respectively (**Figure 4**). Twenty-one species of acylcarnitine in the livers of the pigs were analyzed by ultra-performance LC-MS/MS, and the relative contents of these compounds are shown in Supplemental Table 4. Four of the 21 target species were significantly altered by supranutritional SeMet supplementation. The relative contents of carnitine, hexanoyl-carnitine, decanoyl-carnitine, and tetradecanoyl-carnitine in the Se-S group were 40.4%, 96.4%, 38.7%, and 43.6% greater, respectively, than those in the Se-A group ($P < 0.05$) (**Figure 5**).

Six liver enzymes responsible for sugar and acylcarnitine metabolism were analyzed, and the results are shown in **Figure 6**. The activity of PEPCK in the Se-S group was 64.4% ($P < 0.05$) greater than that in the Se-A group. As expected, the activities of hexokinase and SDH in the Se-S group were 56.7% ($P < 0.05$) and 46.5% ($P < 0.05$) lower, respectively, than that in the Se-A group. The activities of LDH, MDH, and FASN in the liver of pigs did not differ between the 2 groups ($P > 0.05$).

## Discussion

In recent years, the positive correlation between high Se intake and human metabolic disorders (especially type 2 diabetes) has

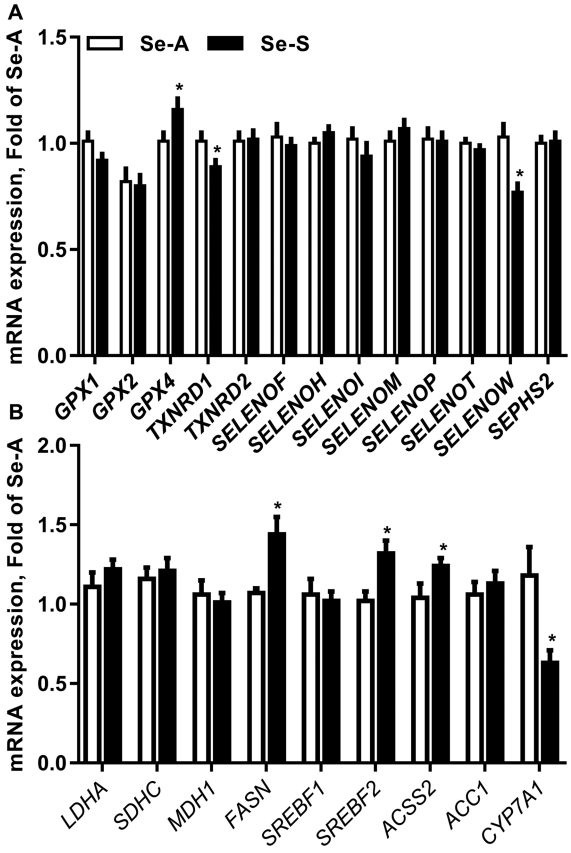


**FIGURE 3** Relative mRNA levels of selenoproteins (A) and genes involved in sugar and lipid metabolism (B) in the liver of pigs fed an Se-A (0.25 mg/kg) and Se-S (2.5 mg/kg) diet for 60 d. Values are means ± SEMs, $n = 6$. *Different from Se-A, $P < 0.05$. *ACC1*, acetyl-CoA carboxylase-1; *ACSS2*, acyl-CoA synthetase short chain family member 2; *CYP7A1*, cytochrome P450, family 7, subfamily A, polypeptide-1; *FASN*, fatty acid synthase; *GPX*, glutathione peroxidase; *LDHA*, lactate dehydrogenase A; *MDH1*, malate dehydrogenase 1; *SDHC*, succinate dehydrogenase complex subunit C; Se-A, selenium adequate; Se-S, selenium supranutritional; *SELENOF*, selenoprotein F; *SELENOH*, selenoprotein H; *SELENOI*, selenoprotein I; *SELENOM*, selenoprotein M; *SELENOP*, selenoprotein P; *SELENOT*, selenoprotein T; *SELENOW*, selenoprotein W; *SEPHS2*, selenophosphate synthetase 2; *SREBF*, sterol regulatory element binding transcription factor; *TXNRD*, thioredoxin reductase.

caused great concern. The recommended daily Se intake for healthy adults is 55 μg, and the maximum daily tolerance of Se is set at 400 μg, ~7.3-fold the requirement (34). Here, we conducted a study in healthy male growing pigs fed either an Se-A (0.25 mg/kg, pig nutrient requirement) or an Se-S (2.5-mg/kg, 10-fold of the requirement) diet with SeMet as the Se source. After 60 d of supplementation, the systemic (serum, liver, muscle, heart, and kidney; Table 1) Se status of pigs in the Se-S group was significantly increased, and the growth performance (Supplemental Table 5) was not significantly affected, as compared with that in the Se-A group. These results are in accordance with findings from previous studies in pigs and rats fed a high Se-yeast diet (3.0 mg Se/kg) (13, 14). In contrast, dietary Se supplementation with 3.0 mg Se/kg (sodium selenite) strongly decreased the growth performance of chickens (19), possibly because of the higher toxicity of inorganic forms of this element (sodium selenite) than organic species (SeMet and Se-yeast) (35) or the different sensitivity

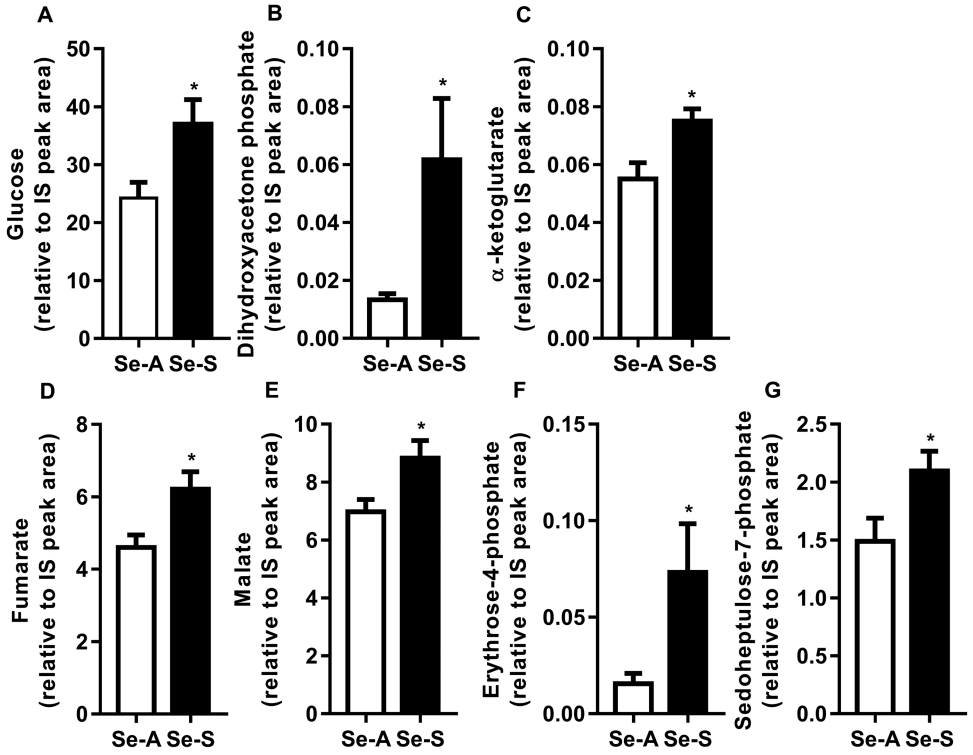


**FIGURE 4** Relative to IS peak area amounts of glucose (A), dihydroxyacetone phosphate (B), α-ketoglutarate (C), fumarate (D), malate (E), erythrose-4-phosphate (F), and sedoheptulose-7-phosphate (G) in the liver of pigs fed an Se-A (0.25 mg/kg) or Se-S (2.5 mg/kg) diet for 60 d. Values are means ± SEMs, $n = 6$. *Different from Se-A, $P < 0.05$. IS, internal standard; Se-A, selenium adequate; Se-S, selenium supranutritional.

to Se among animal species. SeMet, an analog of methionine, is readily incorporated into protein nonspecifically in place of methionine during translation, thus, resulting in dramatic increases in tissue Se in nonruminants supplemented with SeMet compared with inorganic Se, as observed herein and in other studies (36, 37). The increased tissue Se may underlie some of the effects observed in our study.

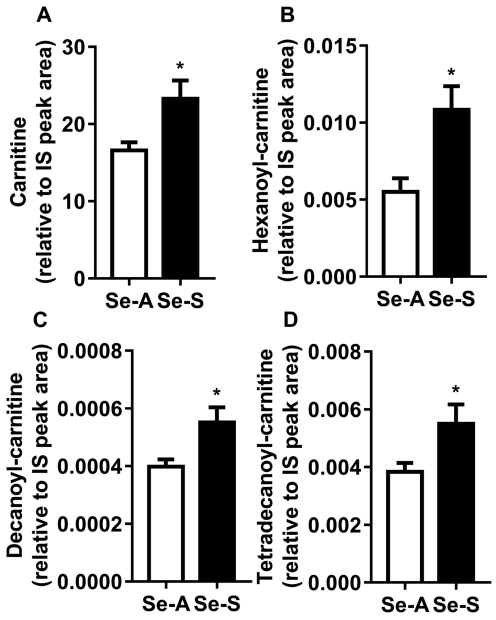


**FIGURE 5** Relative to IS peak area abundance of carnitine (A), hexanoyl-carnitine (B), decanoyl-carnitine (C), and tetradecanoyl-carnitine (D) in the liver of pigs fed an Se-A (0.25 mg/kg) or Se-S (2.5 mg/kg) diet for 60 d. Values are means ± SEMs, $n = 6$. *Different from Se-A, $P < 0.05$. IS, internal standard; Se-A, selenium adequate; Se-S, selenium supranutritional.

Although many parameters (TC, TG, LDL cholesterol, and HDL cholesterol, Supplemental Table 5) in the serum were not significantly affected by high Se treatment, significantly higher amounts of fasting glucose, insulin, and FFAs were observed in the Se-S group than in the Se-A group (Figure 2). Similarly, in rats, prolonged high Se intake (3.0 mg Se/kg), compared with a 0.3 mg Se/kg diet, induces hyperglycemia, hyperinsulinemia, and insulin resistance (14). Moreover, several studies in humans have indicated a positive correlation between serum Se and amounts of lipid (38, 39). Interestingly, hyperglycemia has not been observed in pigs fed a high Se diet (0.5 or 3.0 mg Se/kg, as Se-yeast) for 16 wk (21, 22), possibly because of the differences in Se supplements or doses used in those studies compared with our present work. Although SeMet (accounting for >50%) is the most abundant species in Se-yeast (40), Se-yeast has been reported to contain >60 unique Se species (41). According to these results, the type 2 diabetes-like phenotype caused by high Se intake in pigs appears to be independent of the form of Se used for supplementation.

Several selenoprotein genes, such as *GPX1* and selenoprotein P are considered to be involved in insulin resistance induced by high Se (Se-yeast) intake in Se-A animals (17, 42). A previous report has revealed the capacity of different Se species to regulate selenoprotein expression (43). Our research confirmed previous reports that supranutritional Se supplementation does not substantially affect selenoprotein gene expression, GPX, and TXNRD activity in Se-A animals (21–23). The decreased selenoprotein gene expression levels (*TXNRD1* and *SELENOW*, Figure 3) caused by high Se intake were also in accordance with data from a previous study in pigs (22).

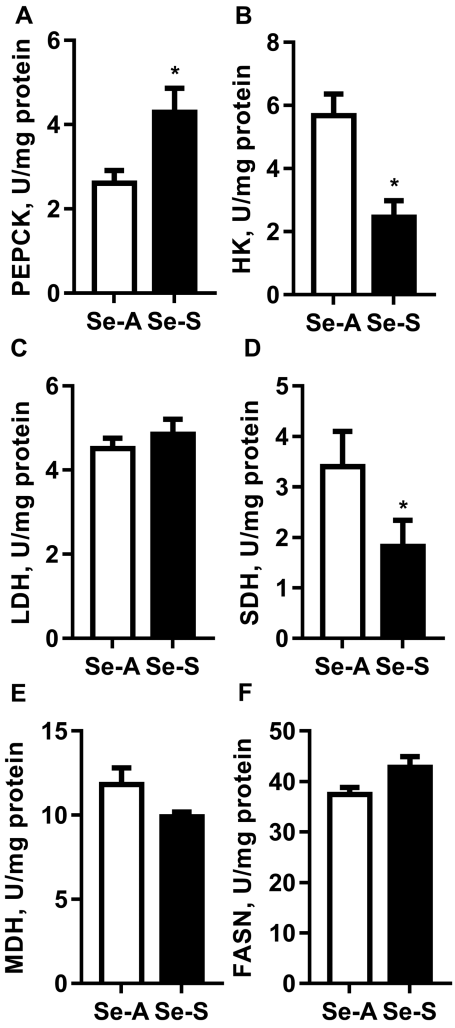


**FIGURE 6** Activity of PEPCK (A), HK (B), LDH (C), SDH (D), MDH (E), and FASN (F) in the liver of pigs fed an Se-A (0.25 mg/kg) or Se-S (2.5 mg/kg) diet for 60 d. Values are means ± SEMs, $n = 6$. *Different from Se-A, $P < 0.05$. FASN, fatty acid synthase; HK, hexokinase; LDH, lactic dehydrogenase; MDH, malate dehydrogenase; PEPCK, phosphoenolpyruvate carboxy kinase; SDH, succinate dehydrogenase; Se-A, selenium adequate; Se-S, selenium supranutritional.

*SELENOW* is considered to exert an important antioxidant function in mammals (44), and its potential role in metabolic disorders induced by high Se intake remains to be further studied. In contrast to our results, high Se (3.0 mg Se/kg, in the form of sodium selenite) intake, compared with 0.15 mg Se/kg intake, has been found to significantly elevate the levels of 7 and 12 selenoprotein mRNAs in the liver and muscle of chickens, respectively (19); these findings may be attributed to different Se sources used for supplementation or different studied animal species. SeMet is an analog of methionine that is readily incorporated into protein nonspecifically in place of methionine, thus potentially explaining why supplementation with SeMet, compared with inorganic Se species, only modestly altered selenoprotein gene expression and selenoenzyme activity (36, 37).

The effects of supranutritional Se supplementation on crucial regulators of sugar and lipid metabolism have been considered a putative mechanism underlying diabetes induced by high Se intake (45). Moreover, several reports have shown that a high Se diet affects the protein amounts of AMP-activated protein kinase, protein kinase B, and mammalian target of rapamycin (21, 22), key regulators in energy metabolism. Glycolysis, the tricarboxylic acid cycle, and the pentose phosphate pathway have important roles in regulating sugar and lipid metabolism, but little is known about the responses of these 3 pathways to supranutritional Se supplementation. Our fundamental goal in the present study was to elucidate the effects of high SeMet intake on sugar and lipid metabolism. We observed that 2 metabolites (glucose and dihydroxyacetone phosphate) in the glycolysis pathway (Figure 4) were more elevated in the Se-S group than the Se-A group, and we also found that the activity of hexokinase was significantly decreased in the Se-S group, whereas the activity of PEPCK was greatly enhanced, by high Se treatment (Figure 6); thus, suggesting that high Se intake suppresses the glycolysis pathway in the liver of pigs. This finding is consistent with findings from previous studies (21, 22) in which a high Se diet changed the amounts of key enzymes in glycolysis (pyruvate kinase) and gluconeogenesis (PEPCK) pathways. Similarly, research in humans has indicated that supranutritional Se supplementation with Se-yeast (200 $\mu$g/day) for 6 wk significantly decreased the expression of the *LDHA* gene in plasma (46). Moreover, high Se supplementation also significantly enhanced metabolites of the tricarboxylic acid cycle ($\alpha$-ketoglutarate, fumarate, and malate) (Figure 4) along with decreasing the SDH and MDH activity (Figure 6). These findings indicated that high Se supplementation might also suppress the tricarboxylic acid cycle pathway in the liver of pigs.

Combined evidence from studies in pigs suggests that supranutritional Se intake enhances *SREBF1*, acetyl-coenzyme A carboxylase, *FASN*, and forkhead box O1 (21, 22), all of which are associated with lipid metabolism. In addition, pigs fed a high Se diet for 16 wk show significant increases in deposition of 7 types of fatty acids in liver tissues (22). Furthermore, in a well-characterized mouse study, supranutritional Se supplementation has been found to alter mitochondrial fatty acid oxidation and glucose homeostasis in the liver (23). Acylcarnitine plays an important role in lipid transport and metabolism, but the effect of high Se supplementation on acylcarnitine metabolism remains unclear. Therefore, we used a targeted method to examine the relative amounts of 21 acylcarnitine compounds. We found that 4 metabolites involved in acylcarnitine metabolism (carnitine, hexanoyl-carnitine, decanoyl-carnitine, and tetradecanoyl-carnitine) (Figure 5) were higher in the Se-S group than the Se-A group. In addition, we observed that the relative expression levels of *FASN*, *SREBF2*, and *ACSS2* in the Se-S group were significantly higher than those in the Se-A group (Figure 3B), suggesting that high Se treatment elevates lipid synthesis. Thus, the improvement in lipid synthesis induced by high Se supplementation may be linked to these elevated acylcarnitine metabolites. In mice (23), carnitine in liver tissues is higher under Se-S than Se-A conditions, in accordance with our findings. These results indicate that high SeMet supplementation induces fatty acid accumulation in the liver of pigs and may be associated with increased acylcarnitine metabolism.

In summary, we examined the effects of supranutritional SeMet supplementation on expression of selenoproteins and genes involved in sugar and lipid metabolism, enzyme activity, and sugar and acylcarnitine metabolism in the liver of pigs. The higher systemic Se status induced by dietary supranutritional SeMet supplementation resulted in diabetes-like phenotypes

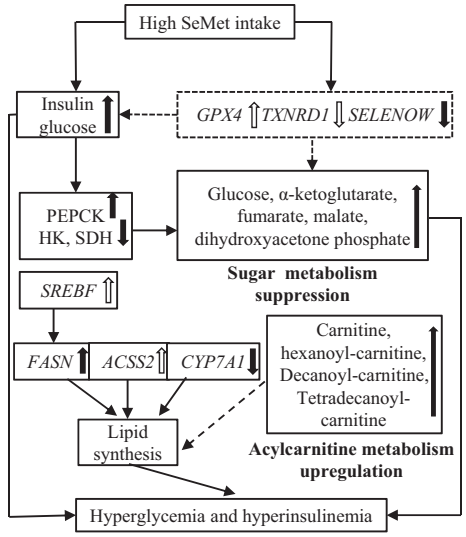


**FIGURE 7** Effects of high Se intake on sugar and acylcarnitine metabolism in the liver of pigs. Solid lines represent data-supported pathways, and dashed lines suggest unsupported pathways. Solid black up/down arrows indicate dramatically altered effects, and outlined arrows indicate smaller effects. *ACSS2*, acyl-CoA synthetase short chain family member 2; *CYP7A1*, cytochrome P450, family 7, subfamily A, polypeptide-1; *FASN*, fatty acid synthase; *GPX4*, glutathione peroxidase 4; HK, hexokinase; PEPCK, phosphoenolpyruvate carboxy kinase; SDH, succinate dehydrogenase; SeMet, selenomethionine; *SELENOW*, selenoprotein W, *SREBF*, sterol regulatory element binding transcription factor; *TXNRD1*, thioredoxin reductase 1.

such as hyperglycemia, hyperinsulinemia, and FFA accumulation in pigs. Our results showed a novel effect of high Se intake on molecular targets related to sugar and acylcarnitine metabolism. As shown in **Figure 7**, dietary high SeMet-induced hyperglycemia and hyperinsulinemia were associated with suppression of sugar metabolism and elevation of lipid synthesis in the liver of pigs.


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