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A high-selenium diet induces insulin resistance in gestating rats and their offspring

Min-Shu Zeng^a, Xi Li^a, Yan Liu^a, Hua Zhao^a, Ji-Chang Zhou^a, Ke Li^a, Jia-Qiang Huang^a, Lv-Hui Sun^a, Jia-Yong Tang^a, Xin-Jie Xia^{a,b}, Kang-Ning Wang^a, and Xin Gen Lei^{a,c,*}

^aInternational Center of Future Agriculture for Human Health, Sichuan Agricultural University, Chengdu, Sichuan, China

^bInstitute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, China

^cDepartment of Animal Science, Cornell University, Ithaca, NY 14853, USA

Abstract

Although supranutrition of selenium (Se) is considered a promising anti-cancer strategy, recent human studies have shown an intriguing association between high body Se status and diabetic risk. This study was done to determine if a prolonged high intake of dietary Se actually induced gestational diabetes in rat dams and insulin resistance in their offspring. Forty-five 67-day-old female Wistar rats ($n=15/\text{diet}$) were fed a Se-deficient (0.01 mg/kg) corn-soy basal diet (BD) or BD+Se (as Se-yeast) at 0.3 or 3.0 mg/kg from 5 weeks before breeding to day 14 postpartum. Offspring ($n=8/\text{diet}$) of the 0.3 and 3.0 mg Se/kg dams were fed with the same respective diet until age 112 days. Compared with the 0.3 mg Se/kg diet, the 3.0 mg/kg diet induced hyperinsulinemia ($P<0.01$), insulin resistance ($P<0.01$), and glucose intolerance ($P<0.01$) in the dams at late gestation and/or day 14 postpartum and in the offspring at age 112 days. These impairments concurred with decreased ($P<0.05$) mRNA and/or protein levels of six insulin signal proteins in liver and muscle of dams and/or pups. Dietary Se produced dose-dependent increases in *Gpx1* mRNA or GPX1 activity in pancreas, liver, and erythrocytes of dams. The 3.0 mg Se/kg diet decreased *Selh* ($P<0.01$), *Sepp1* ($P=0.06$), and *Sepw1* ($P<0.01$), but increased *Sels* ($P<0.05$) mRNA levels in the liver of the offspring, compared with the 0.3 mg Se/kg diet. In conclusion, supranutrition of Se as a Se-enriched yeast in rats induced gestational diabetes and insulin resistance. Expression of six selenoprotein genes, in particular *Gpx1*, was linked to this metabolic disorder.

Keywords

Gestational diabetes; Insulin resistance; Dietary selenium; Selenoprotein; Free radicals

Insulin resistance and diabetes afflict millions of people in the world. As a well-known antioxidant nutrient [1] and anti-cancer agent [2], selenium (Se) has been considered to be an insulin mimic [3] and antidiabetic [4]. Indeed, a number of animal experiments and epidemiologic investigations have shown correlations between Se deficiency and glucose or lipid metabolic impairment [5,6] or low plasma Se or selenoprotein status in diabetic subjects [7]. However, this rather “prevailing” view of Se has been seriously challenged by findings from recent human and animal studies. First, a strong positive correlation between

erythrocyte glutathione peroxidase-1 (GPX1) activity and insulin resistance was reported in pregnant women during late gestation [8]. Then, type 2 diabetes-like phenotypes were seen in mice overexpressing Se-dependent GPX1 [9]. More striking, a post hoc analysis of the Nutritional Prevention of Cancer trial revealed an approximately twofold increase in type 2 diabetes incidence in the Se-supplemented group compared with the placebo group [10] and a hazard ratio of 2.7 in subjects in the highest tertile of plasma Se concentrations. Thereafter, a number of other recent major human studies, including the prematurely terminated SELECT, have shown positive correlations between high body Se status and adverse blood glucose and lipid profiles [11,12], although mixed or positive effects of Se on decreasing diabetic risk have also been demonstrated in other studies [3,4]. Because supranutrition of Se is still considered an effective anti-cancer remedy, it is urgent to clarify if there is a cause–effect role of high Se intake in inducing diabetes or insulin resistance under well-controlled physiological conditions.

As a major type of diabetes, gestational diabetes mellitus (GDM) affects up to 14% of all pregnancies [13]. Metabolically, GDM is manifested as insulin resistance, glucose intolerance, hyperinsulinemia, and hyperglycemia [14]. Insulin resistance is often associated with impaired insulin signaling in insulin-responsive tissues, including liver and muscle. Major signal proteins in the cascade include insulin receptor (IR), insulin substrate-1 (IRS1), insulin substrate-2 (IRS2), serine/threonine protein kinase 2 (AKT2), forkhead box O1 (FOXO1), and peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1). After Se was shown to be an insulin mimic in isolated rat adipocytes [15], a number of studies have shown the ability of selenate to initiate the translocation of glucose transporters to the membrane surface [16] and to induce phosphorylation of the IR [17]. The latter event activates the insulin signaling cascade [18] and allows association of IRS with the regulatory subunit of phosphoinositide 3-kinase (PI3K). PI3K activates 3-phosphoinositide-dependent protein kinase 1, which activates AKT [19]. Mice lacking AKT2 develop insulin resistance and diabetes mellitus-like syndrome [20]. Forkhead transcription factors of the FOXO family are important downstream targets of protein kinase B/AKT [21]. FOXO1 confers insulin sensitivity onto glucose 6-phosphatase expression [22]. Furthermore, PGC-1 binds and coactivates FOXO1 in a manner inhibited by AKT-mediated phosphorylation [23]. Many in vitro and in vivo studies have illustrated that dysregulated expression, localization, and/or activity of any of those proteins may result in insulin resistance [20,23–26].

Meanwhile, the expression and functions of these insulin signal proteins may be affected by Se via redox or other changes [27,28]. A total of 24 or 25 selenoprotein genes [29] have been identified in mammals. Although expression of many selenoprotein genes is downregulated by dietary Se deficiency [30], only a fraction of these genes, including *Gpx1*, *Gpx2*, *Gpx4*, *Selh*, *Seli*, *Selv*, *Sepp1*, *Sepw1*, and *Sepx1*, were affected by dietary Se above the level of nutrient requirement [31,32]. Because GDM in pregnant women is associated with elevated erythrocyte GPX activity [8,33], it is fascinating to investigate if high Se intake induces GDM and insulin resistance by mediating expression of *Gpx1* and all these selected selenoprotein genes in insulin-producing and target tissues. Because of the well-studied Se biology in rats and their relatively short period of gestation, we selected first-parity rat dams to determine: (1) if a prolonged high Se intake induced GDM in the dams and insulin resistance in their offspring and (2) which selenoprotein genes expressed in pancreas, liver, and muscle were associated with the metabolic phenotypes.

Materials and methods

Animals, diet, experimental design, and sample collection

Our animal protocol was approved by Sichuan Agricultural University. A total of 45 female Wistar rats (67 days of age; Experimental Animal Center, Sichuan University, Chengdu,

China) were divided into three groups ($n=15$) and fed a Se-deficient, corn–soybean meal (produced in the Se-deficient area of Sichuan, China) basal diet (BD; 12 μg Se/kg by analysis; Table 1) supplemented with 0, 0.3, or 3.0 mg of Se/kg (as Se-enriched yeast; Angel Yeast, Hubei, China; 1000 mg Se/kg by analysis) for 5 weeks. All three experimental diets were from the same preparation of ingredient mixing (use the BD formulation) to ensure identical concentrations of all nutrients except for Se. The 3 mg Se/kg was achieved by adding the Se-enriched yeast at 3 g/kg directly into the BD, and the 0.3 mg Se/kg was achieved by incorporating the prediluted Se-enriched yeast mixed with ground corn at a ratio of 100:900 (weight, final Se concentration of the diluted form, 100 mg/kg) at 3 g/kg. The 0.3 mg Se/kg meets the nutrient requirement of Se for rats, and the 3.0 mg Se/kg is often used in anti-tumor experiments [34,35]. Thereafter, the rats were bred and killed for blood and tissue sampling at day 14 postpartum. Pregnancy was assumed at the expulsion of a vaginal plug, and the day the plug was found was designated as day 1 of pregnancy. In late pregnancy (e.g., day 16), body weight gain and palpable pups were also used as indications of pregnancy. A total of 24 offspring born to dams fed BD+0.3 mg Se/kg ($n=12$) or BD+3.0 mg Se/kg ($n=12$) were selected based on litter (from multiple dams), body weight (close to the average of the group), and gender (half males and half females). The pups were fed the same diet as their respective dam and killed at age 112 days to collect samples. Rats were housed individually in hanging wire-mesh cages, and the temperature was kept at 21–27 °C with a 12-h light/dark cycle. Animals were provided free access to the designated diets in cups and deionized water in stainless steel troughs. Body weight was measured weekly.

At the selected time points, rats were fasted for 8 h (12:00 to 8:00 AM) and then killed by decapitation to collect blood, liver, pancreas, and the soleus muscle. After being immediately dissected on an ice-cold surface, the liver was perfused with and pancreas and muscle were washed with ice-cold isotonic saline before being minced with surgical scissors. The minced samples were divided into aliquots, snap-frozen in liquid nitrogen, and stored at –80 °C until use. Plasma samples were prepared by centrifugation of the whole blood (sodium EDTA as anticoagulant, 2200 g for 15 min, 5804R centrifuge, F45-30-11 rotor; Eppendorf, Hamburg, Germany) and stored at –80 °C. Red blood cells were washed three times with isotonic saline and stored at –80 °C.

Blood or plasma glucose, insulin, and lipid assays

Fasting blood glucose and plasma insulin concentrations, glucose tolerance test (GTT; 2 g/kg), and insulin tolerance test (ITT; 0.5 U/kg) were determined at various time points. Rats were fasted for 8 h (from 12:00 to 8:00 AM) before GTT or ITT. Whole-blood glucose was determined by clipping tails and using the Glucocard blood glucose test meter (GT-1640; Arkaray, Kyoto, Japan). Plasma insulin was determined by using a rat insulin ELISA kit (Groundwork Biotechnology Diagnostic Ltd., Shanghai, China) according to the manufacturer's instructions. Fasting plasma glucose (FPG) and fasting plasma insulin (FPI) were used to calculate the homeostasis model assessment of insulin resistance index (HOMA-IR): $\text{FPG (mg/dl)} \times \text{FPI (}\mu\text{U/ml)} / 2430$ [36]. Plasma total cholesterol (TC) and triglyceride (TG) concentrations were determined using ELISA kits (Groundwork Biotechnology Diagnostic Ltd.).

Real-time quantitative PCR analysis of selenoprotein and insulin signal protein mRNA levels

To determine the effects of dietary Se on the pancreatic mRNA expression of eight selenoprotein genes (*Gpx1*, *Gpx2*, *Gpx4*, *Selh*, *Selv*, *Sepp1*, *Sepp2*, and *Sepp3*) that were affected by high Se intake based on the literature and our own preliminary data, we isolated total RNA from pancreas (50 mg tissue) of four dams killed at day 14 postpartum per diet group. Total RNA was also isolated from liver and muscle of both dams and offspring ($n=4$ /

diet) killed at the designated time points to assay for the effect of dietary Se on the gene expression of six selenoproteins (*Gpx1*, *Selh*, *Seli*, *Sels*, *Sepp1*, and *Sepp2*) and six insulin signaling-related proteins (*Akt2*, *FoxO1*, *Insr*, *Irs1*, *Irs2*, *Pgc-1*). The RNA sample preparation, Q-PCR procedure, relative mRNA abundance qualification, and data-quality control (Supplementary Figs. 1 and 2) were the same as previously described [30,31]. Primers (Supplementary Table 1) for individual selenoprotein, insulin signal protein, and reference (β -actin (*Actb*) and *Gapdh*) genes were designed using Primer Express 3.0 (Applied Biosystems, Foster City, CA, USA).

Se concentration, GPX activity, and Western blot analyses

Concentrations of Se in feed and the Se-enriched yeast were measured using the hydride generation–atomic fluorescence spectrometer (AFS-830; Titan Instruments, Beijing, China) [37], against a standard reference of Se (GBW (E) 080441; National Research Center for Certified Reference Materials, Beijing, China). GPX activity was measured using a kit (A005; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and an ultraviolet–visible spectrophotometer (DU800; Beckman). Protein concentration was determined using the Bradford method. Western blot analyses of hepatic IR and AKT protein levels in the offspring were conducted as previously-described [65].

Statistical analysis

One-way ANOVA (SPSS for Windows 13.0) was used to test the effects of the dietary Se levels on measures. Multiple mean comparisons were performed using Duncan's test. Data are presented as means $\pm$ SE, and significance level was set at $P < 0.05$.

Results

Dietary Se effects on glucose homeostasis and insulin sensitivity of dams

Body weight, FPG, FPI, and HOMA-IR were similar among the three groups of dams initially (Supplementary Table 2). The 5-week feeding of the experimental diets before breeding did not cause any significant differences among groups in these four measures in rats (Fig. 1, D0ges). However, the 3.0 mg Se group had approximately 60% higher ($P < 0.05$) FPI on day 19 of gestation and 24% higher ($P < 0.05$) FPG on day 14 postpartum than the other two groups, respectively (Fig. 1). Consequently, the 3.0 mg Se group at those time points showed 37 to 59% higher ($P < 0.05$) HOMA-IR index than the other two groups. In addition, the actual body weight gain of the dams between day 0 and day 19 was 8% ($P < 0.05$) greater in the 3.0 mg Se group than in the BD group (Supplementary Table 2), despite no significant differences in average body weight among the three diet groups at any time point.

With virtually identical GTT and ITT curves compared to the other two groups on day 0 of gestation, the 3.0 mg Se group demonstrated ($P < 0.05$) glucose intolerance and insulin resistance on day 19 of gestation (Fig. 2). After an intraperitoneal injection of glucose (Fig. 2B) or insulin (Fig. 2D), the 3.0 mg Se group displayed 25 to 64% higher ($P < 0.05$) blood glucose concentrations at 15, 30, and 60 min than those fed BD or 0.3 mg Se/kg. Plasma triglyceride concentrations were increased ($P < 0.05$) by dietary Se on day 19 of gestation, but the treatment difference disappeared by day 14 postpartum (Supplementary Table 2).

Dietary and maternal Se effects on glucose homeostasis and insulin sensitivity of offspring

The combination of dietary Se treatment and maternal body Se status showed no significant effect on body weight of offspring at the age of 112 days (Fig. 3A), although the 3.0 mg Se group had higher ($P < 0.05$) body weights at age 28 to 56 days than the other group (data not

shown). With almost identical FPG compared to the 0.3 mg Se/kg group (Fig. 3B), the 3.0 mg Se/kg group demonstrated 11% higher ($P<0.05$) FPI (Fig. 3C) and 12% higher ($P<0.05$) HOMA-IR index (Fig. 3D). In agreement, the 3.0 mg Se/kg offspring were glucose intolerant and insulin resistant, compared with the 0.3 mg Se/kg group (Fig. 4). However, these two groups of offspring had similar plasma concentrations of TC (2.2 ± 0.1 vs 2.1 ± 0.1 mmol/L) and TG (1.6 ± 0.1 vs 1.5 ± 0.1 mmol/L).

Dietary Se effects on pancreatic gene expression of selenoproteins in dams

Pancreatic mRNA levels of four of the eight selenoprotein genes assayed were affected ($P<0.05$) by dietary Se concentrations in dams on day 14 postpartum (Table 2). Whereas *Gpx1* mRNA levels increased with dietary Se concentrations, the opposite was true for those of *Seiv*. The *Selh* mRNA level in the 0.3 and 3.0 mg Se groups was 5.2- and 3.7-fold higher ($P<0.05$), respectively, than that of the BD group. The *Sepw1* mRNA level in the 0.3 mg Se group was 2-fold higher ($P<0.05$) than that of the BD group. Elevating dietary Se from 0.3 to 3.0 mg/kg resulted in a 50% decrease in the *Sepw1* and *Seiv* mRNA levels, but the differences were not statistically significant.

Dietary Se effects on liver and muscle gene expression of selenoproteins and insulin signal proteins in dams

Dietary Se supplementation resulted in a dose-dependent decrease ($P<0.04$ to 0.09) in mRNA levels of *Irs1*, *Insr*, and *Akt2* in the liver of dams on day 14 postpartum (Table 3). Hepatic *Irs2* mRNA levels were also 29% lower ($P<0.05$) in the 0.3 mg Se/kg group compared to the BD group, but there was no further decline in the 3.0 mg Se/kg group. In muscle, dietary Se did not affect mRNA levels of any of the six insulin signal proteins except for a difference ($P<0.05$) in *Akt2* between the BD and the 3.0 mg Se/kg groups. Hepatic mRNA levels of *Gpx1*, *Selh*, and *Sepw1* were approximately 50% higher ($P<0.05$) in the two Se-supplemented groups compared to those of the BD group. The same trend was also true for muscle *Selh*, *Sels*, and *Sepw1*. Muscle *Seiv* mRNA levels showed a dose-dependent increase relative to dietary Se concentrations. Meanwhile, GPX activity in erythrocytes of dams on day 19 of gestation and in liver of dams on day 14 postpartum was increased ($P<0.05$) with dietary Se concentration (Fig. 5).

Dietary Se effects on liver and muscle gene expression of selenoproteins and insulin signal proteins in offspring

Compared with the 0.3 mg Se/kg diet, the 3.0 mg Se/kg diet resulted in approximately 50% decreases ($P<0.01$) in mRNA levels of *Akt2*, *Insr*, and *Irs1* and 36% decrease ($P=0.06$) in the mRNA level of *FoxO1* in liver of the offspring (Table 4). In agreement, these decreased hepatic mRNA levels of *Insr* and *Akt2* were verified by approximately 60% decreases in the respective proteins IR and AKT (Figs. 6A and B). Although the mRNA abundance of these genes in muscle was not significantly altered by the high-Se diet, the treatment decreased ($P<0.01$) the expression of *Irs2* and *Pgc-1* by 52 and 36%, respectively. The high-Se diet caused approximately 40% decreases ($P<0.01$) in *Selh* and *Sepw1*, but 36% increase ($P<0.05$) in *Sels* mRNA levels in liver. Hepatic *Sepp1* mRNA was also decreased (18%, $P=0.06$) by the high-Se diet. However, none of the six selenoprotein genes was significantly affected by dietary Se level in muscle. Liver GPX activity in the 3.0 mg Se/kg group was 58% higher ($P<0.05$) than that of the 0.3 mg Se/kg group (Fig. 6C).

Discussion

The most convincing finding from this study is that the high-Se (at 3.0 mg/kg, as Se-enriched yeast) diet indeed induced a moderate GDM and postpartum insulin resistance in the first-parity rat dams. Feeding the Wistar rat dams with this high-Se diet, starting from 5

weeks before breeding and thereafter, produced characteristic phenotypes of GDM in late gestation (day 19). Compared with those fed the other two lower Se diets, these dams showed increased body weight gain, glucose intolerance, insulin resistance, hyperinsulinemia, and elevated HOMA-IR index. Overall, these outcomes suggest that the recently reported increased incidence of type 2 diabetes or adverse blood glucose/lipid profiles associated with high body Se status in humans [7,11,12,38,39] could potentially be caused by elevated dietary Se intake per se. Thus, our findings reveal a realistic risk of prolonged high Se intake and argue against nondiscretionary Se supplements for Se-adequate subjects. Furthermore, plasma triglyceride concentrations were also increased ($P<0.05$) by the high dietary Se intake on day 19 of gestation, although the treatment difference disappeared by day 14 postpartum. This hyperlipidemic (TG) effect of the high-Se diet in the gestating dams was also similar to what was reported previously in weanling albino rats fed supplemental dietary Se from 0 to 2 mg/kg [40,41]. Furthermore, a recent study indicated that C57BL/6 J mice fed a torula yeast-based diet supplemented with Se at 0.4 mg/kg developed hyperinsulinemia and lost insulin sensitivity, compared with the control mice [42]. Reciprocally, disturbing body Se metabolism (reducing selenoprotein synthesis) by overexpressing an i(6)A(-) mutant selenocysteine tRNA promoted glucose intolerance and led to a type 2 diabetes-like phenotype. Apparently, a balanced dietary Se nutrition precluding deficiency or excess is important to avoid the diabetogenic potential of Se. However, dietary Se deficiency did not cause any apparent change in glucose metabolism or insulin sensitivity in the dams of this study during gestation or lactation. Because the main chemical form of Se in the Se-enriched yeast is selenomethionine, which is the major dietary Se compound ingested by people in the United States and elsewhere [1,10,38,39], our findings are quite relevant to public health. Nevertheless, potential confounding effects of yeast constituents other than Se on the outcome may not be completely ruled out even though the inclusion rate of the Se-enriched yeast in the 3 mg Se/kg diet was extremely low (3 g/kg).

It is unique for this study to illustrate the carryover and/or additive effect of the maternal Se status and a continued high-Se intake on postpartum dams and adult offspring. Although dams fed the high-Se diet were no longer hyperinsulinemic on day 14 postpartum, they became hyperglycemic and thus still had higher HOMA-IR index (insulin resistant) than the other two groups. Pups born to them and fed the same high-Se diet for another 16 weeks developed insulin resistance and had greater body weight during the first 8 weeks of age than those fed the 0.3 mg Se/kg diet. These changes were similar to the “giant baby” syndrome associated with maternal GDM [43]. Although this study could not distinguish between the relative contributions of maternal GDM and postnatal continuation of high Se intake, the final metabolic abnormality in the offspring, the overall response, indicates an alarming effect of high Se on glucose metabolism and pediatric health [44]. The insulin resistance induced by the high-Se diet in the dams and offspring was associated with a consistent downregulation of mRNA levels of hepatic *Insr*, *Irs1*, and *Akt2* genes and/or hepatic IR and AKT protein levels. The high Se intake also decreased mRNA levels of hepatic *Irs2* in the dams as well as those of hepatic *FoxO1* and muscle *Irs2* and *Pgc-1* in the offspring. Because these genes code for important insulin signal proteins in the cascade [45], downregulation of their expression or protein production may compromise insulin sensitivity [46]. Early studies have shown downregulation of *Insr*, *Irs1*, and *Akt2* in livers of diabetic animals and humans [20,47–49]. Likewise, the expression of *Insr*, *Irs2*, and *Akt2*, along with several glucose-metabolic-pathway genes, was decreased in pancreatic islets isolated from type 2 diabetic humans [50]. Downregulation of *Insr*, *Irs2*, or *Akt2* led to altered glucose sensing and defective insulin secretion [20,51–53]. Meanwhile, this study showed a declined muscle *Pgc-1* mRNA in the insulin-resistant offspring fed 3.0 mg Se/kg. Our result was consistent with the notion that expression of PGC-1 was reduced in the insulin-resistant compared with insulin-sensitive subjects, and the change was correlated

with those of *Irs1* and *Glut4* [54]. However, responses of *FoxO1* in the postpartum dams and their offspring in the present study were different from those reported in diabetic mouse liver and muscle [23,55,56].

Response profiles of selected selenoprotein genes to dietary Se were determined to explore plausible links between the diabetogenic potential of excess Se and its biochemical forms in vivo. Among all the genes assayed, *Gpx1* mRNA exhibited the most apparent dose-dependent increases with dietary Se concentrations in pancreas and liver of dams. Remarkably, the GPX activity in erythrocytes at late gestation and in liver at day 14 postpartum displayed similar trends of increases. Although these changes resemble the strong positive relationship between erythrocyte GPX activity and the incidence of gestational diabetes and insulin resistance at late pregnancy in humans [8,33], the development of type 2 diabetes-like phenotypes in GPX1-overexpressing mice [9] indicates that overproduction of GPX1 activity diminishes intracellular ROS, resulting in dysregulation of important proteins for insulin synthesis, secretion, and signaling [57]. Similar roles of GPX1 were also reported by two other groups in the high-Se-induced dysregulation of glucose homeostasis in myocytes [58] and mice [42]. Altogether, GPX1 seemed to act as a main mediator of high Se intake in inducing GDM and insulin resistance in this study.

The other five selenoprotein genes, including *Selh*, *Sepp1*, *Sels*, *Selv*, and *Sepw1*, were affected by dietary Se deficiency in pancreas or liver of dams. Although the roles of *Selh*, *Selv*, and *Sepw1* in glucose metabolism or diabetes have not been reported, *Sels* was first isolated during work on diabetic rats and was linked to these events [59,60]. Knockout of *Sepp1* improves insulin sensitivity in mice [61], whereas altered *Sepp1* was seen in patients with type 2 diabetes [62]. It is also interesting to note the reciprocal impact (inhibition) of insulin on transcription of *Sepp1* via the PI3K/AKT/FOXO1 α axis [63], whereas the PGC-1 α -inducing glucocorticoid dexamethasone strongly enhanced *Sepp1* mRNA levels and protein secretion in cultured rat hepatocytes [64]. Although these five selenoprotein genes were shown to be responsive to high Se intake [31], they showed no distinct difference between the 0.3 and the 3.0 mg Se/kg diet groups of dams on day 14 postpartum. The lack of response to the high-Se diet at postpartum might imply a need for us to conduct the assay at late gestation and/or for other target selenoprotein genes in a future study. In contrast, the high-Se diet indeed decreased gene expression of *Selh*, *Sepp1*, and *Sepw1*, but increased *Sels* in liver of offspring at age 112 days, compared with the 0.3 mg Se/kg diet. Because elevation of hepatic *Sels* mRNA was observed in type 2 diabetic *Psammomys obesus* after fasting [60] and rats in the present study were also fasted for 8 h before tissue collection for assays, the upregulation of hepatic *Sels* gene in the offspring might be associated with their insulin resistance. Although the exact functions of *Sels* and other selenoproteins in insulin resistance and glucose metabolism remain to be determined, our results provide direct evidence for their involvement, in addition to GPX1, in the high-Se diet-induced metabolic disorders. Whereas both maximal and deficient expression of selenoproteins could promote development of type 2 diabetes-like phenotypes [42], the impacts of different Se compounds on glucose uptake and insulin signaling in cultured myocytes were more related to their ability to modulate intracellular redox status rather than selenoprotein expression [58]. Alternatively, those selenoproteins that can directly regulate intracellular redox status might serve as main mediators of high Se intake in inducing insulin resistance or diabetes. In this light, it may help explain why overexpression or knockout of GPX1 in mice resulted in such apparent metabolic phenotypes [9,65,66].

Additional considerations should be taken in using our present findings to link expression of specific selenoprotein genes to the high-Se diet-induced gestational diabetes and insulin resistance. Apparently, the pancreatic expression profile of selenoprotein genes detected in

this study was associated with both exo- (acinar) and endocrine (islet) cells in the tissue. Thus, future experiments should isolate islets to determine specific roles of selenoproteins in insulin synthesis and secretion, although our ongoing mouse experiments are showing impacts of Se supranutrition on their gene expression in islets similar to those in rat pancreas of this study. Although many previous studies have suggested that 0.1 to 0.3 mg Se/kg of diet is sufficient to saturate expression of all selenoproteins [1,29,32], dams and pups fed 3.0 mg Se/kg manifested greater levels of GPX activity in liver or erythrocytes than those fed 0.3 mg Se/kg. Seemingly, rats fed the 0.3 mg Se/kg diet might be somewhat Se deficient. However, Se concentrations of the main ingredients, corn and soy; the Se-enriched yeast; and the BD used in this study were determined by actual analysis. Compared with the Se-deficient BD, the 0.3 mg Se/kg diet produced approximately 30-fold increase in liver GPX activity, which was comparable with previous similar studies [30–32]. Meanwhile, mRNA levels of several selenoprotein genes in pancreas, liver, and muscle of dams were also increased by supplementing Se at 0.3 mg/kg into the BD, but showed no further increase or were even downregulated in the 3.0 mg Se/kg group. Using the same batch of Se-enriched yeast, we have demonstrated that 3.0 mg Se/kg enhanced liver GPX activity by 68% ($P<0.05$) in pigs compared with 0.3 mg Se/kg without affecting plasma GPX3 activity [31]. Similar increases in hepatic or erythrocyte GPX activity by high Se over adequate Se supplements have also been seen in mice [67], rats [68], and fish [69]. Thus, the increases in liver and erythrocyte GPX activity in rats fed 3.0 mg Se/kg over those fed 0.3 mg Se/kg were probably a unique mechanism for the supranutrition of Se to induce insulin resistance by creeping the homeostatic regulation of this particular selenoperoxidase [31,67–69].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

Akt2	serine/threonine protein kinase 2
BD	basal diet
FPG	fasting plasma glucose
FPI	fasting plasma insulin
FoxO1	forkhead box O1
Gapdh	glyceraldehyde-3-phosphate dehydrogenase
GDM	gestational diabetes mellitus
Gpx	glutathione peroxidase
HOMA-IR	homeostasis model assessment of insulin resistance
IR/Insr	insulin receptor
Irs	insulin substrate
ITT	insulin tolerance test
Pgc-1	peroxisome proliferator-activated receptor γ , coactivator-1 α

PI3K	phosphoinositide 3-kinase
Q-PCR	real-time quantitative PCR
Selh	selenoprotein H
Seli	selenoprotein I
Sels	selenoprotein S
Selv	selenoprotein V
Sepp1	selenoprotein P
Sepw1	selenoprotein W
SePX1	selenoprotein X
ROS	reactive oxygen species
TC	total cholesterol
TG	triglyceride

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Appendix A

Supplementary data

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.freeradbiomed.2012.01.017.

Fig. 1.

Effects of dietary Se concentration on (A) body weight (BW), (B) fasting plasma glucose concentration (FPG), (C) fasting plasma insulin concentration (FPI), and (D) HOMA-IR of dams on days 0 and 19 of gestation (D0ges and D19ges, respectively) and day 14 postpartum (D14pp). Values are means±SE, $n=7$ to 10. Means that do not share the same superscript letter (a, b) are different ($P<0.05$).

Fig. 2. Effects of dietary Se concentration on (A and B) glucose tolerance test and (C and D) insulin tolerance test in dams on days 0 (A and C) and 19 (B and D) of gestation. Values are means $\pm$ SE, $n=6$. * $P<0.05$; ** $P<0.01$, compared with the BD and 0.3 mg Se/kg groups.

Fig. 3. Effects of dietary Se concentration on (A) body weight (BW), (B) fasting plasma glucose concentration (FPG), (C) fasting plasma insulin concentration (FPI), and (D) HOMA-IR of offspring at age 112 days. Values are means $\pm$ SE, $n=8$. Means showing different superscript letters (a, b) are different ($P<0.05$).

Fig. 4. Effects of dietary Se concentration on (A) glucose tolerance test and (B) insulin tolerance test of offspring at age 112 days. Values are means $\pm$ SE, $n=8$, ** $P<0.01$, compared with the 0.3 mg Se/kg group.

Fig. 5. Effects of dietary Se concentration on GPX activity (A) in erythrocytes of dams on day 19 of gestation and (B) in liver of dams on day 14 postpartum. Values are means $\pm$ SE, $n=5$. Means showing different superscript letter are different ($P<0.05$).

Fig. 6. Effects of dietary Se concentration on relative amounts of (A) IR and (B) AKT and (C) GPX activity in liver of offspring at age 112 days. Values are means $\pm$ SE, $n=3$ (A and B) or 6 (C). Means showing different superscript letter are different ($P<0.05$).

Table 1Composition of BD (as fed)^a.

	Content
Ingredient	
Corn (g/kg)	580.6
Roasted soybean meal (g/kg)	332.6
Soybean oil (g/kg)	60.0
CaCO ₃ (g/kg)	9.2
CaHPO ₄ (g/kg)	3.0
NaCl (g/kg)	2.6
Choline (g/kg)	1.0
Trace mineral premix ^b (g/kg)	0.4
Vitamin premix ^c (g/kg)	1.0
Amino acid premix ^d (g/kg)	9.6
Total (g/kg)	1000.0
Nutrient composition (calculated)	
Metabolism energy (MJ/kg)	15.3
Crude protein (%)	17.8
Lysine (%)	1.3
Methionine (%)	0.5
Methionine+cysteine (%)	0.8
Calcium (%)	0.5
Available phosphorus (%)	0.2

^aThe Se concentrations of BD and of Se-rich yeast (Angel Yeast) were 12 µg/kg and 1000 mg/kg, respectively.

^bTrace mineral premix provided per kilogram of diet: FeSO₄·7H₂O, 194 mg; CuSO₄·5H₂O, 26 mg; ZnSO₄, 150 mg; MnSO₄, 36 mg; and colistin sulfate, 0.15 mg.

^cVitamin premix provided per kilogram of diet: retinyl acetate, 15,000 IU; cholecalciferol, 3000 IU; DL-α-tocopheryl acetate, 57.5 IU; menadione, 31.5 mg; thiamin, 0.6 mg; riboflavin, 4.8 mg; calcium pantothenate, 7.5 mg; niacin, 10.5 mg; pyridoxol, 1.8 mg; folacin, 0.15 mg; and cobalamin, 0.009 mg.

^dAmino acid premix provided per kilogram of diet: L-lysine, 5.0 g; DL-methionine, 2.2 g; L-threonine, 0.5 g; L-isoleucine, 1.9 g.

Table 2

Effects of dietary Se concentration on gene expression of selenoproteins in pancreas of dams at day 14 postpartum.

Gene	BD	0.3 mg Se/kg	3.0 mg Se/kg	P value
<i>Gpx1</i>	581.8±117.5 ^b	3,534.4±694.8 ^{ab}	6,097.0±1,487.3 ^a	0.01
<i>Gpx2</i>	159.1±58.9	105.9±29.9	98.1±35.1	0.57
<i>Gpx4</i>	1,862.2±492.4	1,451.0±392.6	1,761.4±411.4	0.79
<i>Selh</i>	52.5±17.5 ^b	324.5±59.7 ^a	249.2±41.9 ^a	0.00
<i>Selv</i>	0.5±0.1 ^a	0.4±0.1 ^{ab}	0.2±0.1 ^b	0.04
<i>Sepp1</i>	38,791.9±6,963.2	37,682.9±5,181.5	28,989.6±4,792.0	0.45
<i>Sepw1</i>	141.3±17.4 ^b	428.1±110.1 ^a	217.8±48.5 ^{ab}	0.05
<i>Sepx1</i>	1,030.7±157.3	1,064.9±185.3	954.8±48.4	0.86

Values are means±SE, *n*=4. Means in a row without a common letter differ, *P*<0.05.

Table 3

Effects of dietary Se concentration on gene expression of selenoproteins and insulin signal proteins in liver and muscle of dams at day 14 postpartum.

Gene	Liver		P		Muscle			P
	BD	0.3 mg Se/kg	3.0 mg Se/kg		BD	0.3 mg Se/kg	3.0 mg Se/kg	
Insulin signal proteins								
Akt2	552.6±41.8 ^a	528.6±23.0 ^{ab}	453.6±15.0 ^b	0.09	749.8±112.6 ^b	989.4±36.9 ^{ab}	1,044.3±74.0 ^a	0.06
FoxO1	274.2±25.7	314.1±69.7	331.0±38.4	0.70	201.3±33.2	340.6±51.0	396.5±105.2	0.18
Insr	512.0±47.4 ^a	455.0±29.2 ^{ab}	386.5±17.6 ^b	0.08	249.8±77.5	374.7±23.3	345.1±58.7	0.32
Irs1	547.9±31.5 ^a	447.6±59.4 ^{ab}	364.8±32.2 ^b	0.04	548.4±159.8	813.1±152.5	632.5±92.8	0.42
Irs2	271.2±16.7 ^a	192.1±22.0 ^b	233.3±20.2 ^{ab}	0.06	439.8±163.7	431.5±41.9	617.9±169.8	0.58
Pgc-1	140.9±28.9	153.4±25.3	186.8±43.0	0.62	173.1±53.1	297.2±46.4	342.8±78.9	0.18
Selenoproteins								
Gpx1	3,515.5±1,270.6 ^b	6,421.0±490.1 ^a	7,349.7±240.8 ^a	0.02	1,319.0±266.3	2,583.3±635.5	1,980.5±437.8	0.22
Selh	92.2±32.7 ^b	197.7±14.8 ^a	189.2±17.0 ^a	0.02	175.3±44.9 ^b	468.0±40.6 ^a	417.7±39.8 ^a	0.00
Seli	395.6±40.5	362.1±41.4	336.9±27.6	0.55	103.9±18.1	128.7±7.8	121.9±10.8	0.41
Sels	245.4±19.3	223.5±3.4	211.0±13.6	0.25	99.9±18.6 ^b	184.8±19.4 ^a	173.9±15.6 ^a	0.02
Sepp1	12,049.9±958.5	14,384.6±202.2	11,148.4±930.1	0.13	2,425.4±506.2	3,095.0±268.7	3,065.2±212.9	0.36
Sepw1	255.2±118.8 ^b	753.5±39.8 ^a	642.3±63.3 ^a	0.01	3,604.7±1,097.5 ^b	12,042.0±942.2 ^a	12,877.7±1,386.1 ^a	0.00

Values are means±SE, n=4. Means in a row without a common letter differ, $P<0.05$.

Effects of dietary Se concentration on gene expression of selenoproteins and insulin signal proteins in liver and muscle of offspring at day 112 of age.

Table 4

Gene	Liver	P	Muscle		P
	0.3 mg Se/kg		3.0 mg Se/kg	0.3 mg Se/kg	
Insulin signal proteins					
Akt2	554.8±58.2 ^b	0.00	1,506.1±131.4	1,555.2±93.0	0.76
FoxO1	298.2±30.8	0.06	436.1±67.2	329.7±61.6	0.26
Insr	518.9±63.6 ^b	0.00	426.5±28.4	414.8±45.9	0.83
Irs1	813.2±147.1 ^b	0.01	758.7±61.5	808.7±75.5	0.62
Irs2	301.2±47.3	0.60	459.0±58.6 ^b	220±38.3 ^a	0.01
Pgc-1	150.2±16.4	0.28	462.3±69.6 ^b	305.3±43.7 ^a	0.01
Selenoproteins					
Gpx1	5,850.8±389.7	0.58	1,698.6±129.2	1,589.8±110.3	0.53
Selh	428.7±31.0 ^b	0.00	309.3±20.6	282.9±21.7	0.39
Seli	352.8±24.7	0.96	167.3±8.6	195.5±14.8	0.12
Sels	242.2±16.0 ^b	0.02	119.9±11.9	120.5±11.8	0.97
Sepp1	12,057.5±482.0	0.06	2,829.9±172.2	2,740.1±212.8	0.75
Sepw1	322.4±37.4 ^b	0.01	11,305.3±488.7	11,810.7±617.4	0.53

Values are means±SE, n=8. Means in a row without a common letter differ, P<0.05.