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Impacts of Dietary Selenium Deficiency on Metabolic Phenotypes of Diet-Restricted GPX1-Overexpressing Mice

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Abstract

We previously reported a spontaneous development of type 2 diabetes–like phenotypes in glutathione peroxidase-1 (GPX1)-overexpressing (OE) mice. Diet restriction of these mice rescued all their phenotypes, except for hyperinsulinemia and hypersecretion of insulin. This study was to determine whether dietary Se deficiency eliminated these two primary effects of GPX1 overproduction. Forty-seven male OE and wild-type (WT) mice were fed an Se-adequate (0.4 mg Se/kg) or deficient (<0.02 mg Se/kg) diet at 2 to 3 g (full-fed = 5 g) per day from 4 to 12 weeks of age. Although dietary Se deficiency did not rescue the primary phenotypes of the diet-restricted OE mice, it exerted a strong effect ($p < 0.05$) on mRNA or protein levels (or both) of 14 molecules involved in islet insulin synthesis and secretion and hepatic lipogenesis. Dietary Se deficiency exhibited a hypoinsulinemic trend in OE mice and a strong hypolipidemic effect ($p < 0.05$) in the liver of WT mice. Hepatic lipogenesis was attenuated in OE compared with WT mice. In conclusion, diet restriction might be too overwhelming to allow a demonstration of a dietary Se-depletion effect on the OE phenotypes. Full-fed animals could offer a better chance to illustrate such effects and the underlying mechanisms. *Antioxid. Redox Signal.* 14, 383–390.

Introduction

Selenium-dependent glutathione peroxidase-1 (GPX1) is considered to be a major antioxidant intracellular enzyme (11, 25). Because of the relatively low GPX1 activity in islets (17, 30) and the involvement of oxidative stress in the pathogenesis of diabetes (21, 39), overexpression of GPX1 has been promoted as a strategic therapy for diabetes (8, 20, 35, 45). Whereas dysregulation of GPX1 was implicated in developments of diabetes (37) and diabetes-associated disorders (18, 26, 31, 36), increased erythrocyte GPX1 activity was positively correlated with hyperinsulinemia and insulin resistance in pregnant women (9). More paradoxically, we observed that GPX1-overexpressing (OE) mice developed hyperglycemia, hyperinsulinemia, insulin resistance, and obesity at 6 months of age (34). To determine whether these type 2 diabetes–like phenotypes were caused by or confounded with obesity, we used diet restriction (3 vs. 5 g feed/day) in OE mice from 2 to 6 months of age (49). Strikingly, diet restriction eliminated all their phenotypes except for fasting hyperinsulinemia, including hypersecretion of insulin after glucose stimulation (49). Thus, hyperinsulinemia seemed to be the primary effect of GPX1 overproduction.

The trace element Se is a component of 25 selenoproteins (25) and can modulate the expression of other proteins (3). Earlier work also suggested that Se acts as an insulin mimetic (14), and Se deficiency is associated with the incidence of diabetes (13, 23). Administration of Se to streptozotocin-induced diabetic rats restored their glycemic control, largely by modifying the activities of glycolytic and gluconeogenic enzymes, reducing food intake and body weight, and improving insulin sensitivity (1, 2, 46). However, a number of recent human studies showed an alarming hyperglycemic, hyperlipidemic, and prodiabetic effects of Se supplements (4–7, 12, 27, 28, 32, 42, 43). Apparently, the metabolic role and underlying mechanisms of Se or antioxidant enzymes in diabetes are highly controversial. Because GPX1 was the first identified and is the most abundant selenoprotein in the body (11), we were fascinated to determine whether precluding GPX1 overproduction by Se deficiency could rescue hyperinsulinemia in diet-restricted OE mice and affect their related biochemical regulations.

Materials and Methods

Mice, diets, and glucose metabolism tests

In total, 37 weanling (4 weeks old) WT and OE mice ($n = 11$ to 13 for each genotype by diet) were fed Se-adequate (0.4 mg Se/kg, WT^+ and OE^+) or Se-deficient (<0.02 mg Se/kg, WT^- and OE^-) diet at 2 (4 to 6 weeks of age), 2.5 (7 to 8 weeks of age), and 3 (9 to 16 weeks of age) g/day, respectively. Mice were individually reared in plastic cages in an animal room with a constant temperature (22°C) and a 12-h light–dark cycle, and were given free access to distilled water. All mouse experiments

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were approved by the Institutional Animal Care and Use Committee at Cornell University. The OE mice were derived from a B6C3 (C57B1×C3H) hybrid line (Taconic, Germantown, NY) (10), and the experimental diets were torula yeast and sucrose based, as previously described (10, 49). Individual body weights were recorded biweekly after an 8-h overnight fast. Fasting blood glucose concentrations of mice were measured by using a glucometer (Bayer, Elkhart, IN) via tail bleeding every 4 weeks. At 1 week before the end of the study, fasted mice ($n = 5$ to 8 for each genotype by diet) were tested for insulin tolerance (ITT, 0.5 units per kg body weight; Humulin R; Eli Lilly, Indianapolis, IN), glucose tolerance (GTT, 1 g/kg, D-glucose), and glucose stimulated insulin release (GSIS, 1 g/kg) (tests conducted at 2-day intervals). Plasma insulin concentration was determined by using a rat/mouse insulin ELISA kit (Crystal Chem, Downers Grove, IL) (34).

Table 1. Primers for Q-PCR Analysis of Genes in Islets and Liver

Gene	*Full name*	*Forward (5′---3′)* *Reverse (5′---3′)*
Islets		
cFOS	FBJ murine osteosarcoma viral oncogene homologue	ATTTGACTGGAGGTCTGCCTG CACGTTGCTGATGCTCTTGAC
FoxA2	Forkhead box A2	TGCCCCCTACGCCAACATGA TGGCGTGTGTGTAGCTGCGT
Foxo1	Forkhead box O1	GCTGCAATGGCTATGGTAGGA GTCACAGTCCAAGCGCTCAAT
GK1	Glucokinase 1	CTGTTAGCAGGATGGCAGCTT TTTCCTGGAGAGATGCTGTGG
Glut2	Glucose transporter 2	CTCCAGGAAGGGTGCTAAACC TGCTCCCTATCCGTTCTTCAA
HNF1a	hnf1 homeobox a	GTGTAACTGCACAGGAGGCAAA TTCTCACGTGTCCCAAGACCTA
HNF4a	Hepatic nuclear factor 4α	GCCACAGTTTTCCACCAAGAG AAGGAGGACGTCTGCTTCTGA
HPRT	Hypoxanthine-guanine phosphoribosyltransferase	GGCCAGACTTTGTTGGATTTG TGCGCTCATCTTAGGCTTTGT
IRS2	Insulin-receptor substrate 2	GCCTGGGGATAATGGTGACTA TCCATGAGACTTAGCCGCTTC
JunD	Jun protooncogene-related gene D	CGAGCAGCAGCATGCTGAAGA GCCGACCCTGGTTTCAA
NeuroD/Beta2	Neurogenic differentiation 1	GCCCAGCTTAATGCCATCTTT CAAAAGGGCTGCCTTCTGTAA
p53	Transformation-related protein 53	GACCCTGGCACCTACAATGAA GGGTGGATAAATGCAGACAGG
PDX1	Pancreatic and duodenal homeobox factor 1	CTTAACCTAGGCGTCGCACAA GAAGCTCAGGGCTGTTTTTCC
Pregluc	Preproglucagon	TGAGATGAGCACCATTCTGGA TCCGCAGAGATGTTGTGAAGA
INSI	Preproinsulin 1	AGGACCCACAAGTGGAACAA GTGCAGCACTGATCCACAAT
Sur1	Sulfonylurea receptor	TCAACTTGTCTGGTGGTCAGC GAGCTGAGAAAGGGTCATCCA
UCP2	Uncoupling protein 2	GGGTTCATGCCTTCCTTTCT AGATTGGTAGGCAGCCATTAGG
Liver (also GK1 as for islets)		
ACAA1	Acetylcoenzyme A acyltransferase 1	GCTCTACCACGGCTGGAAAC CGACCACTGCATAGGACCTCA
ACC1	Acetyl-coenzyme A carboxylase 1	TGAGGAGGACCGCATTTATC GCATGGGATGGCAGTAAGGT
CYPA71	Cholesterol 7 alpha-hydroxylase	AGCAACTAAACAACCTGCCAGTACTA GTCCGGATATTCAAGGATGCA
FASN	Fatty acid synthase	AAGGCTGGGCTCTATGGATT TGAGGCTGGGTTGATACCTC
F1,6BP	Fructose 1,6-bisphosphatase	CTTGCCATGGATTGTGGTGT TTGATGGCAGGGTCAAAGTC
MCCC1	Methylcrotonyl coenzyme A carboxylase 1	GGAAAATCGGGTATCCTGTTATG AGACTTCTTCGCCTCCCTCC
SREBP1	Sterol regulatory element–binding protein 1	GGAACAGACACTGGCCGAGA GCATAGGGGGCGTCA
SREBP2	Sterol regulatory element–binding protein 2	GCGTTCTGGAGACCATGGA ACAAAGTTGCTCTGAAAACAAATCA

Tissue collection, sample preparation, and biochemical measures

At the end of the study, the four groups of mice ($n = 6$ group) were killed to collect blood, liver, and pancreas, as previously described (49). Islets were isolated from mice by using a standard procedure, with minor modifications (49). Total RNA was prepared from freshly isolated islets (200 per sample) by using Trizol (Invitrogen, Carlsbad, CA), following the manufacturer's instruction. The mRNA levels of pertaining genes in islets and liver were determined by Q-PCR (7900 HT; Applied Biosystems, Foster City, CA). Genes assayed and primer sequences used for each gene are presented in Table 1. The 2^{-ddCt} method was used for the quantification with *HPRT* as a reference gene, and the relative abundance was normalized to the WT^+ mice (as 1). Protein concentrations of seven insulin-related signal molecules in homogenates of islets (400 per sample; $n = 4$ mice per genotype) and two lipogenesis-regulating proteins in the liver were determined as previously described (34, 49). The immunoreactive protein was detected by reacting with appropriate secondary antibody conjugated to horseradish peroxidase and visualized with a chemiluminescent-substrate system (SuperSignal West Pico; Pierce, Rockford, IL). Density of the protein bands on the scanned image was quantified by using Canvas 7 software (Deneba Systems, Miami, FL) and expressed as a relative percentage of the mean of the WT^+. The antibody information is presented in Table 2.

To extract lipid for the assay of total cholesterol (TC) and total triglycerides (TGs), a total of 100 mg of liver was mixed with 1 ml of PBS and homogenized by using a Polytron. After the homogenate was extracted with chloroform:methanol (5:2.5 ml) and centrifuged, the lower layer was transferred into a glass tube, dried under nitrogen gas, and dissolved into 200 μl of ethanol containing 1% Triton. Concentrations of TC and TG were measured by using a kit (Wako Chemicals, Richmond, VA).

Statistical analyses

Data were analyzed by using SAS (release 6.11; SAS Institute, Cary, NC). Dietary Se and genotype effects were tested by two-way ANOVA with or without time-repeated measurements. Data are presented as mean ± SEM, and significance was set at $p < 0.05$.

Table 2. Name, Type, Dilution, and Source of Primary Antibodies

Antibody	*Species*	*Dilution*	*Source*
Catalase	Goat	1:1,000	Santa Cruz Biotechnology (Santa Cruz, CA)
Foxa2	Goat	1:200	Upstate Biotechnology (Lake Placid, NY)
GPX1	Rabbit	1:5,000	Lab Frontier (Seoul, Korea)
NeuroD/Beta2	Rabbit	1:1,000	Santa Cruz Biotechnology (Santa Cruz, CA)
P53	Rabbit	1:1,000	Santa Cruz Biotechnology (Santa Cruz, CA)
PDX1	Rabbit	1:5,000	Dr. Wright (Vanderbilt University)
SREBP-1	Mouse	1:1,000	Santa Cruz Biotechnology (Santa Cruz, CA)
SREBP-2	Mouse	1:1,000	Cayman Chemical Company
UCP2	Goat	1:500	Upstate Biotechnology (Lake Placid, NY)

Results

Dietary Se deficiency effect on metabolic phenotypes

Effects of dietary Se deficiency and genotype on the experimental animals were confirmed by GPX1 protein expression in islets and liver (Fig. 1A) and GPX activity in liver (Fig. 1B). Although a main effect ($p < 0.05$) was noted of genotype but not dietary Se deficiency on body weight (Fig. 2A) and blood glucose concentrations (Fig. 2B), the group mean differences at various times reached significance only between the WT^+ and OE^+ mice for body weight and only between the WT^- and OE^- for blood glucose concentration. However, postmortem plasma insulin concentrations were greater ($p < 0.001$) in both Se-deficient and adequate OE than in the WT (Fig. 2C). Although dietary Se deficiency led to a 27% decrease of plasma insulin in the OE mice, the difference was not statistically significant. No main effect of either genotype or dietary Se deficiency was seen on body glucose (Fig. 3A) or insulin (Fig. 3B) tolerance, except for two notable changes. The first was a lower ($p < 0.05$) blood glucose concentration in the OE^- than in the OE^+ at 30 min after the glucose challenge. The second was a greater decrease ($p < 0.05$) in blood glucose concentration in the OE^- and the OE^+ at 30 min after the insulin challenge (Fig. 3B). Interestingly, three of the five WT^- mice, one OE^+, and one OE^- mouse experienced hypoglycemic shock after the insulin challenge and were given an emergency injection of glucose (data were excluded from the remainder of the ITT). As shown earlier, GSIS was upregulated in the OE mice compared with the WT (Fig. 3C).

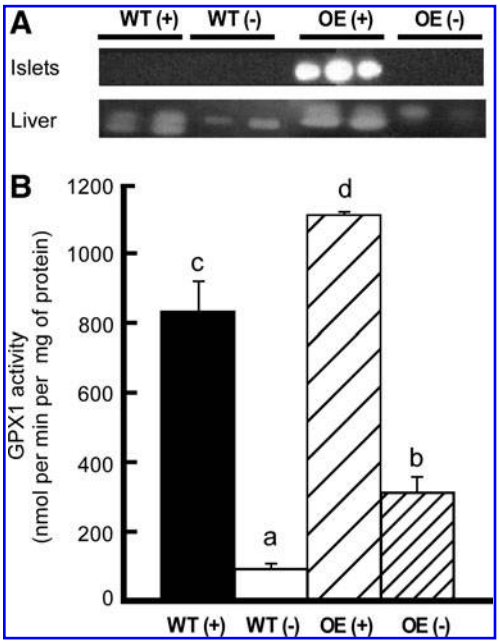

FIG. 1. Effects of dietary Se deficiency on GPX1 protein in islets and liver (A) and GPX activity in liver (B) of WT and OE mice on diet restriction for 12 weeks. Data in (**B**) are expressed as mean ± SEM ($n = 4$ to 7). Values without sharing a common letter differ ($p \leq 0.05$).

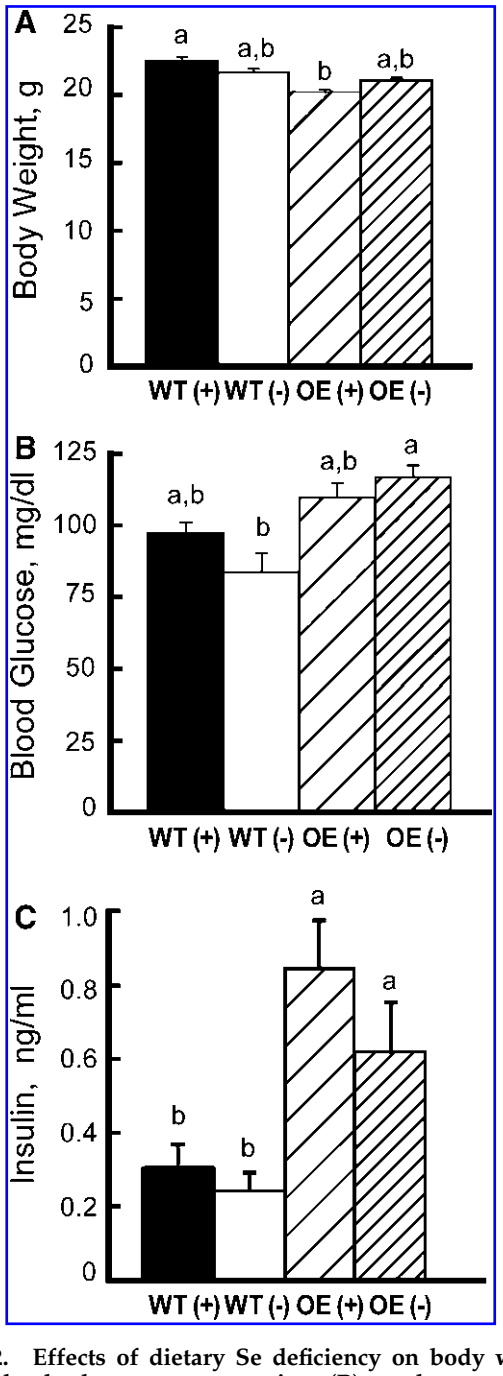

FIG. 2. Effects of dietary Se deficiency on body weight (A), blood glucose concentration (B), and postmortem plasma insulin concentration (C) of WT and OE mice on diet restriction for 12 weeks. Data are expressed as mean ± SEM ($n = 5$ to 13). Values without a common letter differ ($p \leq 0.05$).

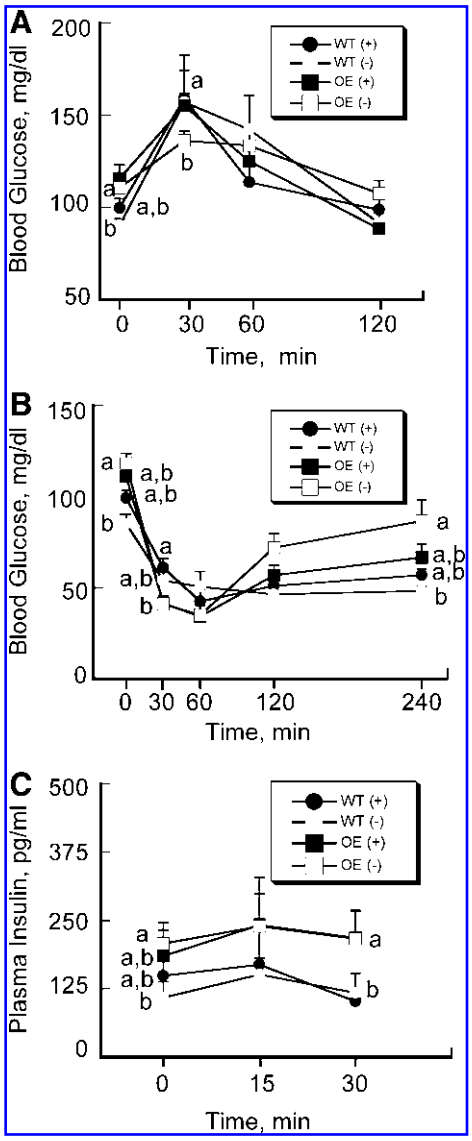

FIG. 3. Effects of dietary Se deficiency on body glucose tolerance (GTT, 1 g/kg) (A), body insulin tolerance (ITT, 0.5 U/kg) (B), and glucose-stimulated insulin secretion (GSIS, 1 g/kg) (C) of WT and OE mice on a diet restriction for 12 weeks. These tests were carried out with mice fasted overnight for 8 h at 1 week before the end of the study (2 days apart for each test). Data are expressed as mean ± SEM ($n = 5$ to 13). Values at a given time without sharing a common letter differ ($p \leq 0.05$).

Dietary Se deficiency effects on islet gene and protein expression

Among the 17 genes assayed in islets (Table 1), nine of them were affected by genotype, dietary Se deficiency, or their interactions (Fig. 4A). Specifically, dietary Se deficiency exerted a main effect ($p < 0.05$) on mRNA levels of *Gk1*, *Pdx1*, *Ucp2*, *Ins1*, and *Pregluc*. Although a main effect ($p < 0.05$) of genotype was noted on mRNA levels of *Pdx1*, *Beta2*, *Glut2*, and *p53*, the latter three genes plus *FoxA2* were affected by a strong interaction ($p < 0.05$) between genotype and dietary Se deficiency. Dietary Se deficiency resulted in a decrease ($p < 0.05$) in *Glut2* and *p53* in only the OE mice, but an increase ($p < 0.05$) in *FoxA2* in only the WT mice, respectively. Western blot analysis indicated an increase of PDX1 protein ($p < 0.05$) and BETA2 protein ($p = 0.09$) in the Se-deficient WT islets compared with their Se controls (Fig. 4B). In contrast, islet p53 or other proteins were not affected by either genotype or dietary Se deficiency.

Dietary Se deficiency effect on hepatic lipogenesis

Liver TC and TG were decreased ($p < 0.05$) by 23% and 41%, respectively, by dietary Se deficiency in the WT mice

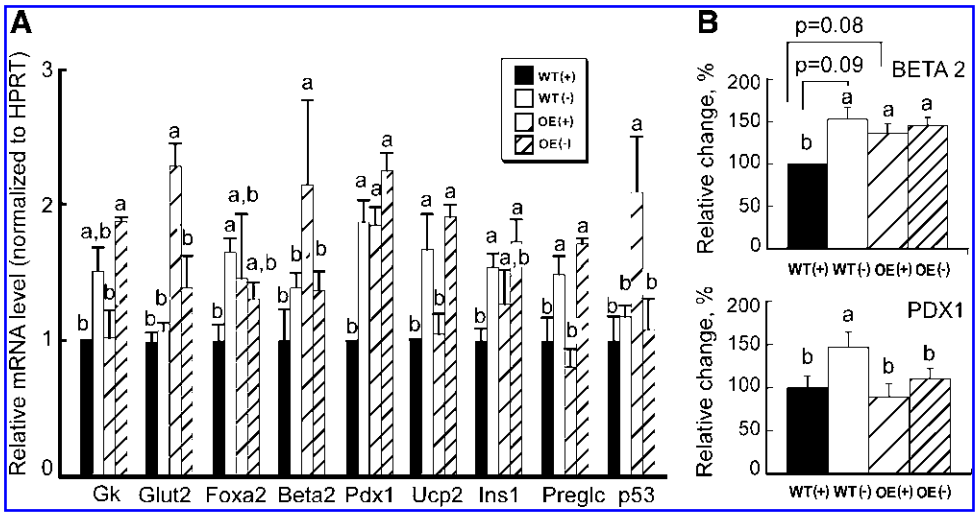

FIG. 4. Effects of dietary Se deficiency on pancreatic islet gene expression (A) and protein expression (B) of WT and OE mice on a diet restriction for 12 weeks. Full lists of primers and antibodies used are presented in Tables 1 and 2, respectively. Data are expressed as mean SEM ($n = 4$ to 7). Values without a common letter differ ($p \leq 0.05$).

(Fig. 5). A similar effect of dietary Se ($p < 0.05$) on liver TC was found in the OE mice. Interestingly, the OE mice had much lower ($p < 0.05$) liver TG than did the WT mice at both levels of dietary Se. Among nine assayed genes related to lipogenesis in the liver (Table 1), three of them were affected ($p < 0.05$) by dietary Se deficiency or genotype (Fig. 6A). Hepatic *FASN* mRNA was more than doubled ($p < 0.05$) in the OE^+ mice than in the WT^+ mice, and was decreased by dietary Se deficiency in the OE mice. The same was also true for hepatic *GK1* mRNA except for that the dietary Se effect in the OE mice just reached marginal significance ($p = 0.06$). A main effect of genotype ($p < 0.05$) was seen in hepatic *F16P* mRNA, and the group mean difference was significant between the WT^+ and OE^+ mice. Despite no changes in their mRNA levels, SREBP-1 and SREBP-2 proteins were downregulated ($p < 0.05$) in the livers of OE mice than in those in the WT^+ (Fig. 6B).

Discussion

The first important finding of the present study is that hyperinsulinemia and elevated GSIS in the diet-restricted OE mice (49) were not eliminated by feeding the Se-deficient diet from 4 to 12 weeks of age. These two disorders are considered to be primary effects of GPX1 overproduction, because diet restriction of the Se-adequate OE mice from 2 to 6 months of age in our previous study failed to reverse these two symptoms when the other type 2 diabetes–like phenotypes, including hyperglycemia, insulin resistance, and obesity, were rescued (49). In the present study, diet restriction in the Se-adequate OE and WT mice exerted effects similar to those in our earlier study on body weight, blood glucose, postmortem plasma insulin concentration, glucose and insulin tolerances, and GSIS. Although the main effect of dietary Se deficiency on these measures was not statistically significant, a few notable points were found. First, dietary Se deficiency minimized the genotype difference in body weights, but amplified such a difference in blood glucose concentrations. Second, dietary Se deficiency seemed to decrease postmortem plasma insulin levels in the OE mice. Third, dietary Se deficiency appeared to improve glucose and insulin tolerances in the OE mice at 30 min after the respective challenges, although a hyperglycemic recovery was seen at 240 min after the insulin challenge. Thus, an involvement of dietary Se in the development of hyperinsulinemia should not be completely ruled out by this study. Plausibly, a longer time than 12 weeks might be needed to show the effects of dietary Se deficiency if the OE metabolic phenotypes are not predisposed or predetermined in early life. Diet restriction might be too overwhelming to allow dietary Se to affect the overt phenotypes, so that animals given free access to diet could be a better model for the purpose. Furthermore, 25 selenoproteins exist in rodents (25). Reducing expressions of certain selenoproteins by dietary Se deficiency

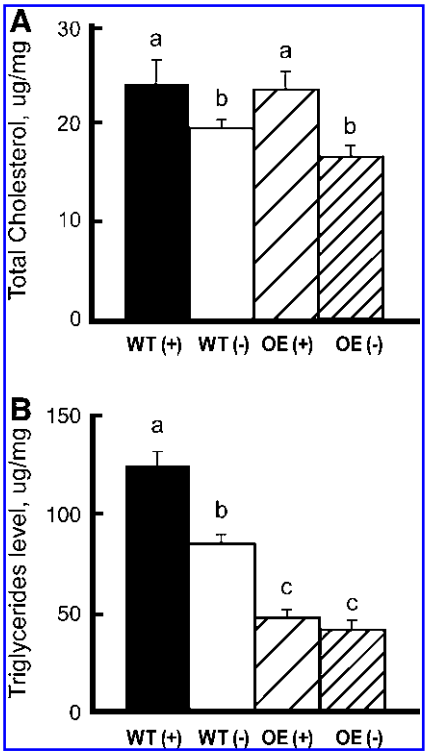

FIG. 5. Effects of dietary Se deficiency on hepatic total cholesterol content (A) and total triglyceride content (B) of WT and OE mice on a diet restriction for 12 weeks. Data are expressed as mean ± SEM ($n = 6$ to 7). Values without a common letter differ ($p \leq 0.05$).

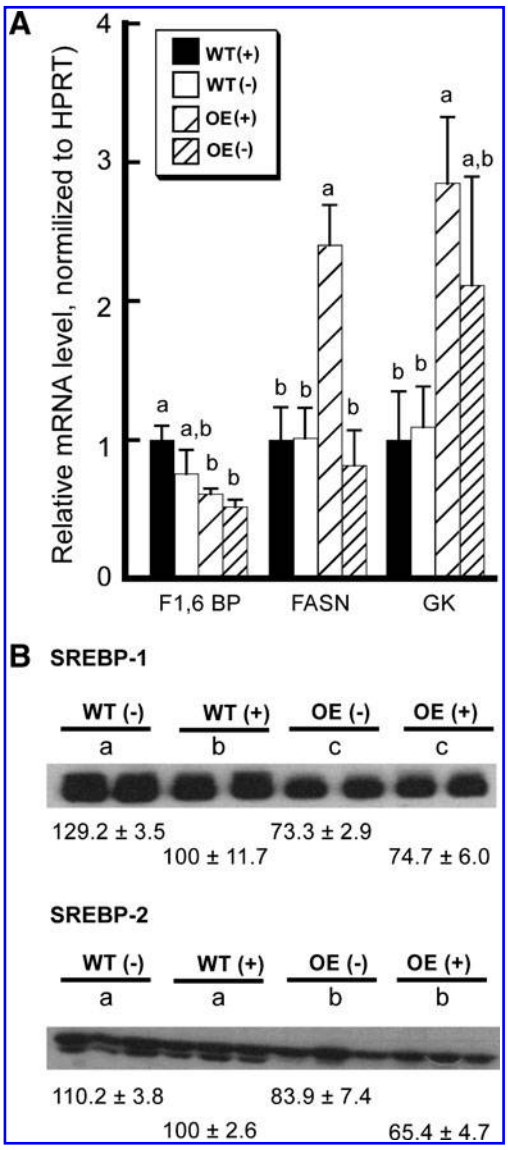

FIG. 6. Effects of dietary Se deficiency on hepatic lipogenesis-related gene expression (A) and SREBP protein (B) of WT and OE mice on a diet restriction for 12 weeks. Full lists of primers and antibodies used are presented in Tables 1 and 2, respectively. Data are expressed as mean ± SEM ($n = 4$ to 7). Values without a common letter differ ($p \leq 0.05$).

(11, 44) could offset any potential effect or benefit of down-regulation of GPX1.

It is novel to show that at least nine genes in islets were affected to a great extent by dietary Se deficiency, in either both or one of the two genotypes. Despite a hypoinsulinemic trend, dietary Se deficiency actually induced expression of "pro-insulin synthesis" genes including *Beta2*, *Foxa1*, *Pdx1*, and *Ins1* (24) and suppressed expression of the *p53* gene. This paradoxic discrepancy may be interpreted by (a) a complex feedback or posttranscriptional mechanism in regulating insulin synthesis; (b) a too-strong diet-restriction effect (19) that overrode the metabolic role of the altered gene expression; and (c) a parallel upregulation of counterreacting genes, such as *preglucagon*. Whereas islet *Beta2* mRNA levels correlated well with the protein in both genotypes, it is hard for us to explain why PDX1 protein in OE islets was not in accordance with its mRNA abundance. Likewise, dietary Se deficiency promoted expression of *Gk1*, *Ucp2*, and *Pregluc* in both genotypes and decreased expression of *Glut2* in the OE islets. Although these four factors play an important role in GSIS (33, 50), the elevated GSIS in the OE mice was not affected by the changes of these genes. As in the case of insulin synthesis discussed earlier, a complex posttranscriptional regulation, a too-potent diet-restriction effect, and a balanced change between the positive (*e.g.*, *GK1*) and negative (*e.g.*, *UCP2*) regulators of GSIS might help explain the disconnection between gene expression and metabolic phenotypes.

Strikingly, the diet-restricted OE mice had lower hepatic TG than did the WT mice, irrespective of dietary Se levels. In consistent, the OE mice exhibited a lower body weight and a decreased amount of hepatic SREBP-1 and SREBP-2 proteins. Because we observed more than double the body fat in the obese OE (Se-adequate) mice than in the WT mice (34), it is intriguing to see an actual lower body weight and down-regulated lipogenesis in these hyperinsulemic animals that were supposed to be lipogenic. As key transcriptional activators for lipogenesis (48), SREBP-1 and SREBP-2 translocate to nucleus and bind to gene-promoter regions of rate-limiting enzymes such as HMG-CoA reductase for cholesterol synthesis (16) and FASN in fatty acid synthesis (29, 41, 47) activate their transcriptions. Although HMG-CoA reductase mRNA was not measured in the present study, responses of liver FASN mRNA to GPX1 transgene or dietary Se deficiency were not correlated with or rather were opposite to the hepatic TG profile. The relative mRNA levels of *GK1* and *F16P*, indicators of glycolysis (15), might represent an increased catabolism of carbohydrate, leading to loss of a carbon source for fat synthesis and loss of body weight. Furthermore, dietary Se deficiency caused a significant decrease in hepatic TC and TG in the WT mice. This hypolipidemic action reconciles an opposite effect of high-Se status on human blood-lipid profiles (7, 28, 42). However, hepatic SREBP-1 or SREBP-2 protein was not altered by dietary Se deficiency in either genotype. Seemingly, overproduction of GPX1 in early life derails body carbohydrate metabolism and lipogenesis from the normal regulatory pathways. A chronic hyperinsulinemia might induce upregulation of counterreactive hormones, such as glucagon, cortisol, and epinephrine (22, 38, 40), shifting anabolism to catabolism. When the diet was restricted, these OE mice were less efficient than the WT in converting food energy into body fat or mass.

In summary, dietary Se deficiency did not reverse hyperinsulinemia or elevated GSIS in the diet-restricted OE mice. The overwhelming metabolic effect of diet restriction and the length of Se deficiency might have precluded such a potential for dietary Se depletion. However, dietary Se deficiency exerted a strong effect on gene and protein expressions involved in insulin synthesis and secretion, as well as lipogenesis. Full-fed OE mice might offer a better chance to illustrate the metabolic role and underlying mechanism of dietary Se in the development of type 2 diabetes–like phenotypes.

Acknowledgment

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

Beta2 = neurogenic differentiation 1
F16P = fructose 1,6-biphosphatase
FASN = fatty acid synthase
Foxa2 = forkhead box A2
GK = glucokinase
GPX1 = cellular glutathione peroxidase-1
$GPX1^{-/-}$ = GPX1 knockout
GSIS = glucose-stimulated insulin secretion
HMG-CoA reductase = 3-hydroxy-3-methyl-glutaryl-CoA reductase
INS1 = preproinsulin 1
IP = immunoprecipitation
OE = GPX1 overexpressing
OE^{+} = OE fed Se-adequate diet
OE^{-} = OE fed Se-deficient diet
p53 = transformation-related protein 53
PBS = phosphate-buffered saline
PDX1 = pancreatic and duodenal homeobox factor 1
Pregluc = preproglucagon
SREBP = sterol regulatory element–binding protein
TC = total cholesterol
TG = total triglycerides
UCP2 = uncoupling protein 2
WT = wild type
WT^{+} = WT fed Se-adequate diet
WT^{-} = WT fed Se-deficient diet

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