High selenium intake and increased diabetes risk: experimental evidence for interplay between selenium and carbohydrate metabolism

Holger Steinbrenner, 1 Bodo Speckmann, 1 Antonio Pinto 1 and Helmut Sies 1,2,3,*

1 Institute for Biochemistry and Molecular Biology I, Medical Factory, Heinrich-Heine-Universität, Düsseldorf, Universitätsstrasse 1, Geb. 22.03, D-40225 Düsseldorf, Germany
2 Institut für Umweltmedizinische Forschung (IUF) an der Heinrich-Heine-Universität, Auf’m Hennekamp 50, D-40225 Düsseldorf, Germany
3 College of Science, King Saud University, Riyadh, Saudi Arabia

(Received 20 July, 2010; Accepted 10 September, 2010)

The essential trace element selenium has long been considered to exhibit anti-diabetic and insulin-mimetic properties, but recent epidemiological studies indicated supranutritional selenium intake and high plasma selenium levels as possible risk factors for development of type 2 diabetes, pointing to adverse effects of selenium on carbohydrate metabolism in humans. However, increased plasma selenium levels might be both a consequence and a cause of diabetes. We summarize current evidence for an interference of selenium compounds with insulin-regulated molecular pathways, most notably the phosphoinositide-3-kinase/protein kinase B signaling cascade, which may underlie some of the pro- and anti-diabetic actions of selenium. Furthermore, we discuss reports of hyperinsulinemia, hyperglycemia and insulin resistance in mice overexpressing the selenoenzyme glutathione peroxidase 1. The peroxisomal proliferator-activated receptor gamma coactivator 1α represents a key regulator for biosynthesis of the physiological selenium transporter, selenoprotein P, as well as for hepatic glucose homeostasis. As proliferator-activated receptor gamma coactivator 1α has been shown to be up-regulated in livers of diabetic animals and to promote insulin resistance, we hypothesize that dysregulated pathways in carbohydrate metabolism and a disturbance of selenium homeostasis are linked via proliferator-activated receptor gamma coactivator 1α.

Key Words: selenoprotein, glutathione peroxidase, hyperglycemia, insulin, PGC-1α, Akt

The essential trace element selenium is believed to exert beneficial influence on human health, mainly based on the antioxidant capacity of selenoproteins such as glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs) containing the antioxidant capacity of selenoproteins such as glutathione peroxidase 1. The peroxisomal proliferator-activated receptor gamma coactivator 1α represents a key regulator for biosynthesis of the physiological selenium transporter, selenoprotein P, as well as for hepatic glucose homeostasis. As proliferator-activated receptor gamma coactivator 1α has been shown to be up-regulated in livers of diabetic animals and to promote insulin resistance, we hypothesize that dysregulated pathways in carbohydrate metabolism and a disturbance of selenium homeostasis are linked via proliferator-activated receptor gamma coactivator 1α.

Anti-Diabetic and Insulin-Mimetic Actions of Selenium

Diabetes mellitus is affecting over 170 million people worldwide with more than 90% of the patients suffering from type 2 diabetes. The onset of type 2 diabetes is hallmarkated by resistance of liver, skeletal muscle and fat tissue to insulin, thereby causing dyslipidemia, hyperglycemia and a reactive increase in insulin secretion by pancreatic beta cells for compensation of the poor insulin response of major target tissues. Binding of insulin to its receptor initiates the intracellular insulin signalling cascade, whose components have been reviewed comprehensively elsewhere. Among them, the insulin receptor substrate (IRS)-
2, the protein tyrosine phosphatase (PTP)-1B and the protein kinase B (serine/threonine kinase Akt) as well as the forkhead box class (Fox) O1a transcription factor and its coactivator peroxisomal proliferator-activated receptor gamma coactivator (PGC)-1α have received particular attention in diabetes research.

At present, it is evident from in vitro and in vivo studies that dysregulated expression, localisation and/or activity of one or more of these proteins may result in insulin resistance. 

Besides selenium, a number of metal ions (e.g., vanadium, copper, zinc and cadmium) are capable of eliciting insulin-mimetic effects by activation of Akt and other kinases of the insulin signaling cascade such as p70 S6 kinase. The insulin-like phosphorylation of Akt upon exposure of cells to micromolar doses induced a cytoprotective response (10 µM) doses of heavy metal ions at oxidation number +II (Cu²⁺, Zn²⁺, Cd²⁺) is interpreted primarily as a stress response, because signaling through phosphoinositide-3-kinase (PI3K) and Akt also promotes anti-apoptotic and cytoprotective pathways. 

With regard to regulation of carbohydrate metabolism, insulin-mimetic properties of selenium compounds at oxidation numbers +IV (sodium selenite) and +VI (sodium selenate) have been reported in close resemblance to such effects of vanadium at oxidation number +IV (vanadyl sulphate). Early studies have been performed in isolated rat adipocytes, and found that sodium selenate stimulated glucose uptake through translocation of glucose transporters to the plasma membrane and activated serine/threonine kinases including the p70 S6 kinase. As these insulin-like actions were observed only at the very high dose of 1 mM sodium selenate, an anti-diabetic application in humans appears to be difficult or impossible. The results of animal studies are somewhat conflicting: A cautionary view is corroborated by a study in genetically obese Zucker rats, whose glucose tolerance was transiently improved during acute selenate exposure, rapidly followed by progressive development of hyperglycemia indicating toxicity of high selenate doses. On the other hand, whole-body insulin sensitivity was improved in type 2 diabetic db/db mice by dietary supplementation with supranutritional sodium selenate doses.

Moreover, sodium selenite effectively improved glucose homeostasis in streptozotocin-treated rodents. Streptozotocin causes necrosis of pancreatic beta cells through DNA alklylation and, to a minor extent, generation of nitric oxide and reactive oxygen species (ROS), resulting in insulin deficiency and hyperglycemia. The anti-diabetic effects of selenite in streptozotocin-treated rats were attributed to partial reversal of abnormal expression and activity of glycolytic and gluconeogenic liver enzymes, whereas plasma insulin levels did not increase upon selenate administration.

Similar to heavy metal ions, sodium selenite at low micromolar doses induced a cytoprotective response in vitro, thereby counter-acting apoptotic cell death following serum withdrawal or exposure to hydrogen peroxide (H₂O₂); survival of both HuH7 hepatoma cells and HIT1080 fibrosarcoma cells was mediated through selenite-induced Akt activation. An insulin-like action of selenite on carbohydrate metabolism was observed in the isolated perfused rat liver, where glucose-stimulated glycogen of break-down was inhibited by infusion of 10 µM sodium selenite. Consistent with the narrow therapeutic range of selenium, higher doses of selenite (500 µM) severely impaired the metabolic function of the liver, causing degeneration and necrosis of periportal hepatocytes. In vivo, oral selenite administration failed to improve insulin sensitivity in type 2 diabetic db/db mice, presumably due to formation of different intermediary selenium metabolites in peripheral organs compared to sodium selenate.

**Adverse Effects of Selenium on Insulin Secretion and Signalling**

An anti-diabetic impact of dietary selenium supplementation would be expected, given both the long track record of selenium as insulin-mimetic micronutrient and its antioxidant capacity as constituent of ROS-detoxifying selenoenzymes, suggesting a protective role against oxidative stress-related chronic complications in the progression of diabetes. 

Contrary to those expectations, recent epidemiological and intervention studies revealed a surprising association between high plasma selenium levels and type 2 diabetes, hyperglycemia and dyslipidemia. 

The clue to answer the pivotal question of whether and how selenium exerts adverse effects on insulin-regulated metabolic pathways in humans may lie in the apparent “redox paradox” of insulin signalling, a concept that refers to facilitated insulin action by insulin-stimulated reactive oxygen species. 

Upon binding to its receptor at the plasma membrane of adipocytes, insulin elicits a transient burst of ROS (superoxide and H₂O₂). Insulin activates the NAD(P)H oxidase (Nox) 4 to generate superoxide, which is subsequently converted to H₂O₂. These insulin-stimulated small amounts of H₂O₂ serve as second messengers, which attenuate the activity of phosphatases with redox-sensitive cysteine residues and thereby enhance the phosphorylation of components downstream in the insulin signalling cascade. Thus, high supranutritional doses of antioxidants may have the capability to impair insulin sensitivity, as it has recently been shown in humans.

Inorganic and organic selenium compounds have been reported to induce expression and activity of several antioxidant selenoproteins; the most pronounced stimulation was obtained for the selenoenzyme cytosolic GPx1, which degrades H₂O₂ and other hydroperoxides. A high GPx1 activity has been hypothesized to interfere with insulin signalling. Indeed, pregnancy-associated mild insulin resistance was shown to be accompanied by increased erythrocyte GPx activity in humans, and transgenic mice overexpressing GPx1 developed at older age a type 2 diabetes-like phenotype characterised by insulin resistance, hyperglycemia, hyperinsulinemia and obesity. GPx1 overexpression affected both pancreatic insulin production and insulin sensitivity of target cells; insulin resistance of liver and/or skeletal muscle was obvious from impaired insulin receptor and Akt phosphorylation. Intriguingly, obesity together with insulin resistance and hyperglycemia could be prevented in the GPx1-overexpressing mice by dietary restriction, whereas the chronic hyperinsulinemia persisted, even at dietary selenium deficiency. The authors conclude that dysregulation of pancreatic insulin biosynthesis and secretion is the primary outcome of transgenic GPx1 overproduction in their experimental model. Insulin-producing pancreatic beta cells are among the worst-endowed cells in terms of intrinsic enzymatic antioxidants: expression and activity of the H₂O₂-degrading enzymes catalase and GPx1 in beta cells reach only 1% of the values in hepatocytes. For this reason, beta cells are very susceptible to damage caused by hyperglycemia or proinflammatory cytokines, and overexpression of antioxidant enzymes including GPx1 has been applied to protect insulinoma cell lines and pancreatic islets from oxidative injury. 

On the other hand, development of hyperinsulinemia in GPx1 overexpressing mice points to detrimental effects of high GPx1 activity on beta cell function in vivo, impairing the tight control of insulin release. An adverse effect of high GPx1 activity on components of the insulin signalling cascade has been further substantiated by an in vitro study in MCF-7 human breast cancer cells, where GPx1 overexpression was associated with decreased phosphorylation of p70 S6 kinase and Akt. An alternative approach to increase GPx1 in a more physiological manner was done by dietary supplementation of rats with sodium selenate; the higher GPx1 activity in livers of selenium-supplemented rats was associated with increased activity of protein tyrosine phosphatase 1B (PTP-1B), which antagonizes insulin-induced signaling by dephosphorylation of the insulin receptor (IR) and the IRS-1.

Conversely and in good agreement with the experimental
models of GPx1 overexpression, knock-out of GPx1 in mice resulted in improved insulin sensitivity due to increased ROS generation, causing oxidation (inactivation) of the dual specificity protein phosphatase PTEN. PTEN dephosphorylates the product of PI3K, phosphatidylinositol-3,4,5-triphosphate (PIP3), thus counteracting insulin-induced PI3K/Akt signalling. In line with elevated PI3K/Akt signaling, insulin-induced glucose uptake was increased in skeletal muscles of GPx−/−mice, and most compelling, knock-out of GPx1 protected the rodents from insulin resistance provoked by high-fat diet. These results are supported by observations of increased site-specific phosphorylation of both Akt and p70 S6 kinase in transgenic mice with an overall decreased biosynthesis of selenoproteins, caused by a mutant form of selenocysteine transfer RNA (tRNA[Ser]Sec). Despite the compelling evidence from transgenic animal models of GPx1 overexpression and knock-down, results from intervention studies with selenium supplements in several human populations argue against the idea that glutathione peroxidases are the only mediators of adverse effects of high dietary selenium intake under physiological conditions: plasma GPx activity in humans has been found to be saturated at selenium dietary supplement doses and total plasma selenium levels well below the values associated with increased risk for type 2 diabetes. Human plasma contains selenium in form of the selenoenzyme GPx3, a low-molecular-weight selenium pool and most notably the selenium transporter selenoprotein P (SeP), which accounts for 50–60% of circulating selenium. Compared to GPx activity, both SeP and the remaining non-selenoprotein plasma selenium pool require a higher dietary selenium intake for their optimization and saturation. It is tempting to speculate that SeP and/or low-molecular-weight selenium compounds may affect insulin-induced signalling pathways related to carbohydrate and lipid metabolism. Fig. 1 schematically summarizes current experimental evidence and hypotheses concerning an influence of selenium on the insulin signalling cascade.

**PGC-1α: a Molecular Switch Linking Selenium and Carbohydrate Metabolism**

The epidemiological association between high plasma selenium levels and hyperglycemia might also be explained by a disturbance of selenium homeostasis as side-effect of a dysregulated carbohydrate metabolism. The major fraction of total selenium in human plasma is present as SeP, which is mainly secreted by the liver and supplies peripheral tissues with selenium. SeP represents a suitable biomarker for selenium status, because its plasma concentration increases in response to different dietary forms and to a wide range of doses in selenium supplementation studies. This obvious importance of SeP for selenium homeostasis prompted us to investigate the regulation of hepatic SeP production by factors related to carbohydrate metabolism. In the human SeP promoter, we identified a motif consisting of a binding site for the FoxO1a transcription factor, located in close proximity to a binding site for hepatocyte nuclear factor 4α (HNF-4α). This motif is conserved in the SeP promoters of humans, rats and mice, and it mediates high-level expression of SeP in the
liver as well as the hormonal regulation of hepatic SeP transcription. Both transcription factors are co-activated by the PGC-1α, which acts as “molecular switch” in response to hormones such as insulin, glucagon and glucocorticoids, well-known for their control of hepatic glucose production and blood glucose levels. Insulin inhibited SeP transcription via the PI3K/Akt/FOXO1a axis, whereas the PGC-1α-inducing glucocorticoid dexamethasone strongly enhanced SeP mRNA levels and protein secretion in cultured rat hepatocytes. Oral administration of dexamethasone has been reported to give rise to a redistribution of selenium in mice, causing a decrease of liver GPx in favor of elevated plasma selenium levels these earlier results can be explained by enhanced hepatic secretion of SeP induced by dexamethasone treatment.

The complex between FOXO1a and its coactivator PGC-1α is of crucial importance for transcriptional regulation of the glucoen-rogenic enzymes glucose-6-phosphatase (G6Pase) and phospho-enolpyruvate carboxykinase (PEP-CCK). Our observation that the selenium transporter SeP is regulated virtually like a glucoen-rogenic enzyme provides a rationale for the hypothesized link between selenium and carbohydrate metabolism. Moreover, PGC-1α is elevated in livers of animal diabetes models, and has been demonstrated to promote insulin resistance. A vicious circle is observed when diabetes is not treated accurately: high glucose up-regulates expression of PGC-1α and glucoenogenic enzymes in the liver, resulting in overproduction of hepatic glucose and increased hyperglycemia. We cultivated rat hepatocytes in the presence of high glucose (25 mM), and found an increase in SeP production paralleled by elevated PGC-1α mRNA levels.

Thus, elevated hepatic PGC-1α may trigger not only hyperglycemia, but also a disturbance in selenium homeostasis. The anti-hyperglycemic drug metformin is widely described for treatment of type 2 diabetes, because it suppresses hepatic glucose production and improves peripheral insulin sensitivity. In parallel with glucoenogegenesis, metformin attenuated hepatic biosynthesis and secretion of SeP in vitro, which might decrease selenium bioavailability in extrahepatic tissues and thereby impair expression and activity of selenoenzymes in vivo. This idea is supported by a study of Pavlovic et al.: A two-week metformin treatment resulted in decreased GPx activity in erythrocytes of obese patients with type 2 diabetes.

Acknowledgments

This work was supported by Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany (STE 1782/2-1, SFB 575/B4). H. Sies is a Fellow of the National Foundation for Cancer Research (NFCR), Bethesda, MD.

Abbreviations

GPx glutathione peroxidase
TrxR thioredoxin reductase
NPC Nutritional Prevention of Cancer
LDL low-density lipoprotein
IRS insulin receptor substrate
PTP protein tyrosine phosphatise
FoxO forkhead box class O
PGC peroxisomal proliferator-activated receptor gamma coactivator
PI3K phosphoinositide-3-kinase
ROS reactive oxygen species
H2O2 hydrogen peroxide
NOX NAD(P)H oxidase
IR insulin receptor
PIP3 phosphatidylinositol-3,4,5-triphosphate
SeP selenoprotein P
HNF-4α hepatocyte nuclear factor 4α
G6Pase glucose-6-phosphatase
PEP-CCK phosphoenolpyruvate carboxykinase

References

1 Steinbrenner H, Sies H. Protection against reactive oxygen species by seleno-proteins. Biochim Biophys Acta 2009; 1790: 1478–1485.
2 Salonen JT, Altlhan G, Huttunen JK, Pikkarainen J, Buska P. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. Lancet 1982; 2: 175–179.
3 Clark LC, Combs GF Jr., Turnbull BW, and et al. Effects of selenium supplementation on the incidence of type 2 diabetes: a randomized trial. Nutritional Prevention of Cancer Study Group. JAMA 1996; 276: 1957–1963.
4 Beck MA, Levander OA, Handy J. Selenium deficiency and viral infection. J Neurochem 2003; 86: 1–12.
5 Chen J, Berry MJ. Selenium and selenoproteins in the brain and brain diseases. J Neurochem 2003; 86: 1–12.
6 Breunisen P, Steinbrenner H, Sies H. Selenium, oxidative stress, and health aspects. Mol Aspects Med 2005; 26: 256–267.
7 Yang G, Yin S, Zhou R, and et al. Studies of safe maximal daily dietary Se-intake in a seleniferous area in China. Part II: Relation between Se-intake and the manifestation of classical signs and certain biochemical alterations in blood and urine. J Trace Elem Electrolytes Health Dis 1989; 3: 123–130.
8 Whanger P, Vendeland S, Park YC, Xia Y. Metabolism of subtoxic levels of selenium in animals and humans. Ann Clin Lab Sci 1996; 26: 99–113.
9 Rayman MP. Food-chain selenium and human health: emphasis on coenzyme Q10. Br J Nutr 2008; 100: 254–268.
10 Stranges S, Marshall JR, Natarajan R, and et al. Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. Ann Intern Med 2007; 147: 217–223.
11 Bleys J, Navas-Acien A, Guallar E. Serum selenium and diabetes in US adults. Diabetes Care 2007; 30: 829–834.
12 Bleys J, Navas-Acien A, Stranges S, Menke A, Miller ER 3rd, Guallar E. Serum selenium and serum lipids in US adults. Am J Clin Nutr 2008; 88: 416–423.
13 Laclaustra M, Navas-Acien A, Stranges S, Ordovas JM, Guallar E. Serum selenium concentrations and diabetes in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. Environ Health Perspect 2009; 117: 1409–1413.
14 Lippman SM, Klein EA, Goodman PJ, and et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2009; 301: 39–51.
15 Laclaustra M, Stranges S, Navas-Acien A, Ordovas JM, Guallar E. Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. Atherosclerosis 2010; 210: 643–648.
16 Stranges S, Laclaustra M, Ji C, Cappuccio FP, and et al. Higher selenium status is associated with adverse blood lipid profile in British adults. J Nutr 2010; 140: 81–87.
17 Akbaraly TN, Arnaud J, Rayman MP, and et al. Plasma selenium and risk of dysglycemia in an elderly French population: results from the prospective Epidemiology of Vascular Ageing Study. Nutr Metab (Lond) 2010; 7: 21.
18 Kornhauser C, Garcia-Ramirez JR, Wrobel K, Perez-Luque EL, Garay-Sevilla ME, Wrobel K. Serum selenium and glutathione peroxidase concentrations in type 2 diabetes mellitus patients. Prim Care Diabetes 2008; 2: 81–85.
19 Mueller AS, Mueller K, Wolf NM, Pallauf J. Selenium and diabetes: an enigma? Free Radic Res 2009; 43: 1029–1059.
20 Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047–1053.
21 Schinner S, Scherbaum WA, Bornstein SR, Barthel A. Molecular mecha-nisms of insulin resistance. Diabet Med 2005; 22: 674–682.
22 Barthel A, Klotz L.O. Phosphoinositide 3-kinase signaling in the cellular response to oxidative stress. Biol Chem 2005; 386: 207–216.
Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 2008; 51: 1515–1524.

Pepper MP, Vatamaniuk MZ, Van X, Roneker CA, Lei X. Impacts of Dietary Selenium Deficiency on Metabolic Phenotypes of Diet-Restricted GPX1-Overexpressing Mice. *Antioxid. Redox Signal* 2010; DOI:10.1089/ars.2010.3295.

Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* 1997; 46: 1734–1742.

Lortz S, Tiedge M, Nachtweg T, Karlsen AE, Nerup J, Lenzen S. Protection of insulin-producing RINm5F cells against cytokine-mediated toxicity through overexpression of antioxidant enzymes. *Diabetes* 2000; 49: 1123–1130.

Myoore TB, Shinkel TA, Collins J, and et al. Overexpression of glutathione peroxidase with two isoforms of superoxide dismutase protects mice islets from oxidative injury and improves islet graft function. *Diabetes* 2005; 54: 2109–2116.

Naar MA, Fedele MJ, Esser K, Diamond AM. GPx-1 modulates Akt and P70S6K phosphorylation and Gadd45 levels in MCF-7 cells. *Free Radic Biol Med* 2004; 37: 187–195.

Mueller AS, Klaussman SD, Wolf NM, and et al. Redox regulation of protein tyrosine phosphatase 1B by manipulation of dietary selenium affects the triglyceride concentration in rat liver. *J Nutr* 2008; 138: 2328–2336.

Cheng A, Dubé N, Gu F, Tremblay ML. Coordinated action of protein tyrosine phosphatases in insulin signal transduction. *Eur J Biochem* 2002; 269: 1050–1059.

Loh K, Deng H, Fukushima A, and et al. Reactive oxygen species enhance insulin sensitivity. *Cell Metab* 2009; 10: 260–272.

Maebara T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem* 1998; 273: 13375–13378.

Hornberger TA, McLoughlin TJ, Leszczynski JK, and et al. Selenoprotein-deficient transgenic mice exhibit enhanced exercise-induced muscle growth. *J Nutr* 2003; 133: 3091–3097.

Duffield AJ, Thomson CD, Hill KE, Williams S. An estimation of selenium requirements for New Zealanders. *Am J Clin Nutr* 1999; 70: 896–903.

Xia Y, Hill KE, Byrne DW, Xu J, Burk RF. Effectiveness of selenium supplements in a low-selenium area of China. *Am J Clin Nutr* 2005; 81: 829–834.

Hurst R, Armah CN, Dainty JR, and et al. Estimating optimal selenium status: results of a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* 2010; 91: 923–931.

Xia Y, Hill KE, Li P, and et al. Optimization of selenoprotein P and other plasma selenium biomarkers for the assessment of the selenium nutritional requirement: a placebo-controlled double-blind study of seleniummethione supplementation in selenium-deficient Chinese subjects. *Am J Clin Nutr* 2010; 92: 525–531; DOI: 10.3945/ajcn.109.092474.

Burk RF, Early DS, Hill KE, Palmer IS, BogoJin ME. Plasma selenium in patients with cirrhosis. *Hepatology* 1998; 27: 794–798.

Schomburg L, Schweizer U, Holtmann B, Flohi L, Sendtner M, Köhrle J. Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues. *Biochem J* 2003; 370: 397–402.

Walter PL, Steinbrenner H, Barthel A, Klotz LO. Stimulation of selenoprotein P promoter activity in hepatoma cells by FoxO1a transcription factor. *Biochem Biophys Res Commun* 2008; 365: 316–321.
71 Speckmann B, Walter PL, Alili L, and et al. Selenoprotein P expression is controlled through interaction of the coactivator PGC-1alpha with FoxO1a and hepatocyte nuclear factor 4alpha transcription factors. Hepatology 2008; 48: 1998–2006.

72 Yoon JC, Puisyserver P, Chen G, and et al. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature 2001; 413: 131–138.

73 Rhee J, Inoue Y, Yoon JC, and et al. Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. Proc Natl Acad Sci USA 2003; 100: 4012–4017.

74 Watanabe C, Kim CY, Satoh H. Tissue-specific modification of selenium concentration by acute and chronic dexamethasone administration in mice. Br J Nutr 1997; 78: 501–509.

75 Massillon D, Barzilai N, Chen W, Hu M, Rossetti L. Glucose regulates in vivo glucose-6-phosphatase gene expression in the liver of diabetic rats. J Biol Chem 1996; 271: 9871–9874.

76 Speckmann B, Sies H, Steinbrenner H. Attenuation of hepatic expression and secretion of selenoprotein P by metformin. Biochem Biophys Res Commun 2009; 387: 158–163.

77 Hundal RS, Kssak M, Dufour S, and et al. Mechanism by which metformin reduces glucose production in type 2 diabetes. Diabetes 2000; 49: 2063–2069.

78 Hundal HS, Ramlal T, Reyes R, Leiter LA, Klip A. Cellular mechanism of metformin action involves glucose transporter translocation from an intracellular pool to the plasma membrane in L6 muscle cells. Endocrinology 1992; 131: 1165–1173.

79 Pavlović D, Kocić R, Kocić G, and et al. Effect of four-week metformin treatment on plasma and erythrocyte antioxidative defense enzymes in newly diagnosed obese patients with type 2 diabetes. Diabetes Obes Metab 2000; 4: 251–256.