

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

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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 1 $\alpha$  represents a key regulator for biosynthesis of the physiological selenium transporter, selenoprotein P, as well as for hepatic gluconeogenesis. As proliferator-activated receptor gamma coactivator 1 $\alpha$  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 1 $\alpha$ .

**Key Words:** selenoprotein, glutathione peroxidase, hyperglycemia, insulin, PGC-1 $\alpha$ , 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 (GPx) and thioredoxin reductases (TrxR) containing the 21<sup>st</sup> proteinogenic amino acid, selenocysteine, in their active center.<sup>(1)</sup> Potential selenium-mediated health benefits include prevention of cardiovascular and neurodegenerative diseases, delay of aging, functioning of the immune system, and prevention of certain forms of cancer.<sup>(1–6)</sup> A wide range of dietary selenium sources comprise cereals, garlic, brazil nuts, meat and fish. Even though overt selenium deficiency is observed rarely, consumers in industrialised countries habitually ingest high amounts of selenium-enriched dietary supplements. However, it has long been known that the therapeutic window of selenium is narrow, and adverse health effects may occur due to supranutritional selenium intake even below the levels required for intoxication.<sup>(7–9)</sup>

In this regard, an ongoing discussion on the safety of dietary selenium supplementation has arisen from a coincidental and unexpected finding of the Nutritional Prevention of Cancer (NPC) trial: participants of the trial, who received a daily dose of 200  $\mu$ g

selenium over 12 years, were more likely to develop type 2 diabetes mellitus than those assigned to placebo.<sup>(10)</sup> Moreover, the diabetes risk of the participants increased with higher baseline plasma selenium levels.<sup>(10)</sup> Since then several epidemiological studies have reported that high plasma selenium levels were associated with increased prevalence of type 2 diabetes as well as hyperglycemia and enhanced plasma levels of total and low-density lipoprotein (LDL) cholesterol and triacylglycerols in the selenium-replete US-American population.<sup>(11–15)</sup> On the other hand, the outcome of similar recent studies in Europe was rather ambiguous, ranging from adverse to slightly beneficial effects of selenium on carbohydrate and/or lipid metabolism.<sup>(16–18)</sup> These divergent results might be explained by a generally lower selenium intake in most European countries in comparison to the USA, but differences in lifestyle and genotype between US-American and European populations as well as varied dietary selenium sources may also contribute.<sup>(9,19)</sup> Given the rising numbers of patients suffering from morbid obesity and diabetes as well as increasing world-wide trends in dietary selenium supplementation, the molecular mechanisms underlying a potential adverse effect of selenium compounds on carbohydrate and/or lipid metabolism need to be addressed. An important unresolved issue, which cannot be answered by epidemiological studies, is the cause-and-effect-relationship of those associations: does selenium oversupply contribute to development of type 2 diabetes by disturbing insulin signalling and/or secretion, or conversely, may a dysregulated carbohydrate metabolism influence selenium homeostasis? Experimental evidence on these issues is available, and to make the picture more complex and somewhat paradoxical, selenium may act as an insulin-mimetic under certain circumstances.

## 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.<sup>(20)</sup> The onset of type 2 diabetes is hallmarked 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.<sup>(21)</sup> Binding of insulin to its receptor initiates the intracellular insulin signalling cascade, whose components have been reviewed comprehensively elsewhere.<sup>(21–23)</sup> Among them, the insulin receptor substrate (IRS)-

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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)-1 $\alpha$  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 those proteins may result in insulin resistance.<sup>(24-28)</sup>

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 (10  $\mu$ M, 100  $\mu$ M) doses of heavy metal ions at oxidation number +II (Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>) is interpreted primarily as a stress response, because signaling through phosphoinositide-3-kinase (PI3K) and Akt also promotes anti-apoptotic and cytoprotective pathways.<sup>(29)</sup> 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).<sup>(30-32)</sup> 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.<sup>(31,33)</sup> 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 cautious 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.<sup>(34)</sup> 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.<sup>(35)</sup> Moreover, sodium selenate effectively improved glucose homeostasis in streptozotocin-treated rodents.<sup>(36,37)</sup> Streptozotocin causes necrosis of pancreatic beta cells through DNA alkylation and, to a minor extent, generation of nitric oxide and reactive oxygen species (ROS), resulting in insulin deficiency and hyperglycemia.<sup>(38)</sup> The anti-diabetic effects of selenate 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.<sup>(37)</sup>

Similar to heavy metal ions, sodium selenite at low micromolar doses induced a cytoprotective response *in vitro*, thereby counteracting apoptotic cell death following serum withdrawal or exposure to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>); survival of both Huh7 hepatoma cells and HT1080 fibrosarcoma cells was mediated through selenite-induced Akt activation.<sup>(39,40)</sup> An insulin-like action of selenite on carbohydrate metabolism was observed in the isolated perfused rat liver, where glucagon-stimulated glycogen breakdown was inhibited by infusion of 10  $\mu$ M sodium selenite.<sup>(32)</sup> Consistent with the narrow therapeutic range of selenium, higher doses of selenite (500  $\mu$ M) severely impaired the metabolic function of the liver, causing degeneration and necrosis of periportal hepatocytes.<sup>(32)</sup> *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.<sup>(35)</sup>

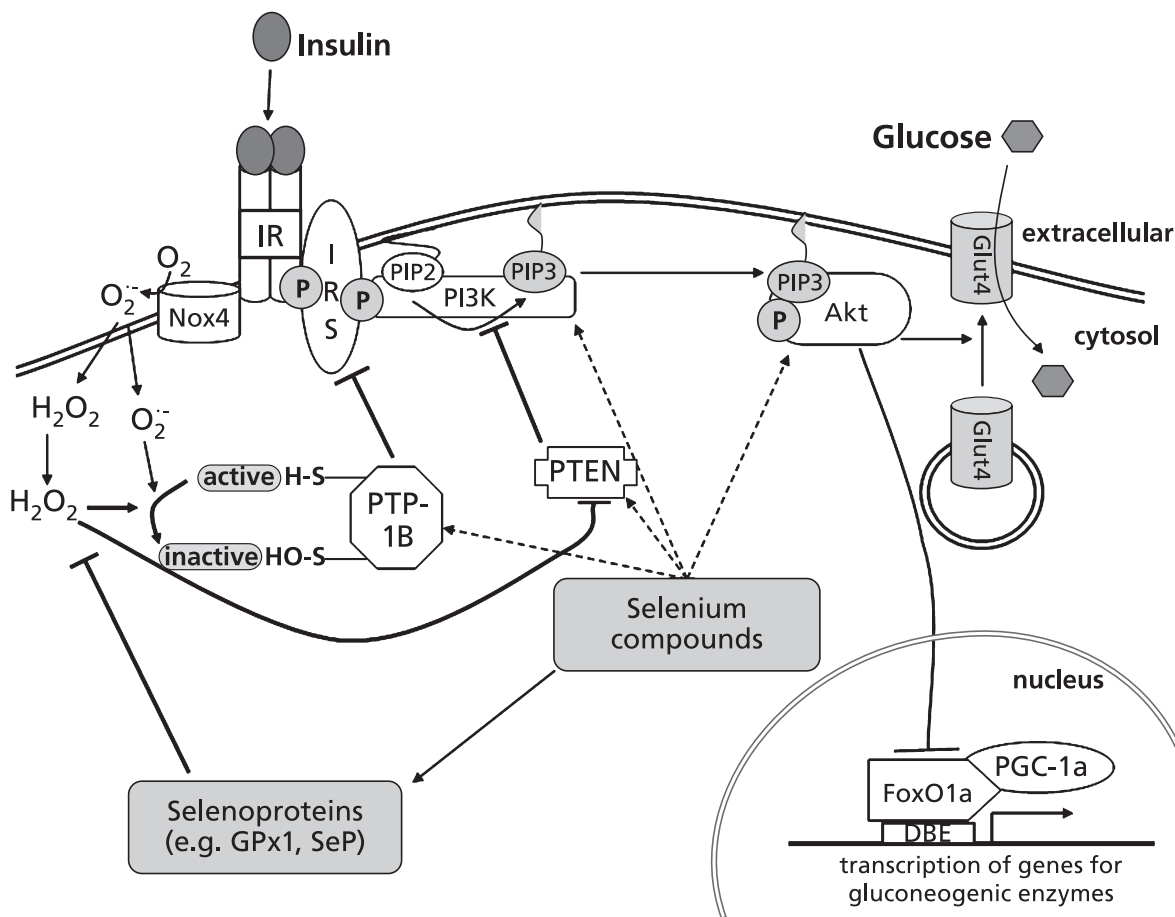
### Adverse Effects of Selenium on Insulin Secretion and Signalling

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.<sup>(1,19,41)</sup> Contrarily to those expectations, recent epidemiological and intervention studies revealed a surprising association between high plasma selenium levels and type 2 diabetes, hyperglycemia and dyslipidemia.<sup>(10-16)</sup> 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.<sup>(42)</sup> Upon binding to its receptor at the plasma membrane of adipocytes, insulin elicits a transient burst of ROS (superoxide and H<sub>2</sub>O<sub>2</sub>).<sup>(43)</sup> Insulin activates the NAD(P)H oxidase (Nox) 4 to generate superoxide, which is subsequently converted to H<sub>2</sub>O<sub>2</sub>.<sup>(44)</sup> These insulin-stimulated small amounts of H<sub>2</sub>O<sub>2</sub> 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.<sup>(42,45)</sup> Thus, high supranutritional doses of antioxidants may have the capability to impair insulin sensitivity, as it has recently been shown in humans administered a combination of vitamin C (1,000 mg/day) and vitamin E (400 IU/day).<sup>(46)</sup>

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,<sup>(47-49)</sup> which degrades H<sub>2</sub>O<sub>2</sub> and other hydroperoxides.<sup>(50)</sup> A high GPx1 activity has been hypothesized to interfere with insulin signaling. Indeed, pregnancy-associated mild insulin resistance was shown to be accompanied by increased erythrocyte GPx activity in humans,<sup>(51)</sup> and transgenic mice overexpressing GPx1 developed at older age a type 2 diabetes-like phenotype characterised by insulin resistance, hyperglycemia, hyperinsulinemia and obesity.<sup>(52)</sup> 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.<sup>(52)</sup> 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.<sup>(53,54)</sup> The authors conclude that dysregulation of pancreatic insulin biosynthesis and secretion is the primary outcome of transgenic GPx1 overproduction in their experimental model.<sup>(54)</sup> Insulin-producing pancreatic beta cells are among the worst-endowed cells in terms of intrinsic enzymatic antioxidants: expression and activity of the H<sub>2</sub>O<sub>2</sub>-degrading enzymes catalase and GPx1 in beta cells reach only 1% of the values in hepatocytes.<sup>(55)</sup> 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.<sup>(56,57)</sup> 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.<sup>(52-54)</sup> 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.<sup>(58)</sup> 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),<sup>(59)</sup> which antagonizes insulin-induced signaling by dephosphorylation of the insulin receptor (IR) and the IRS-1.<sup>(60)</sup>

Conversely and in good agreement with the experimental



**Fig. 1.** Scheme depicting a potential influence of selenium on components of the insulin signaling cascade. Selenoproteins and low molecular weight selenium compounds may interfere at different stages with insulin-induced signal transduction, eventually leading to dysregulation of carbohydrate metabolism. Please see text for details and explanation of the abbreviations.

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 PTP-1B.<sup>(61)</sup> PTP-1B dephosphorylates the product of PI3K, phosphatidylinositol-3,4,5-triphosphate (PIP3), thus counteracting insulin-induced PI3K/Akt signalling.<sup>(62)</sup> In line with elevated PI3K/Akt signaling, insulin-induced glucose uptake was increased in skeletal muscles of GPx<sup>-/-</sup> mice, and most compelling, knock-out of GPx1 protected the rodents from insulin resistance provoked by high-fat diet.<sup>(61)</sup> 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<sup>[Ser]</sup> Sec).<sup>(63)</sup>

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.<sup>(64-67)</sup> 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.<sup>(68)</sup> 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.<sup>(64-67)</sup> 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-1 $\alpha$ : 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.<sup>(68,69)</sup> 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.<sup>(64-67)</sup> 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 4 $\alpha$  (HNF-4 $\alpha$ ).<sup>(70,71)</sup> 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-1 $\alpha$ , which acts as “molecular switch” in response to hormones such as insulin, glucagon and glucocorticoids,<sup>(27,72,73)</sup> well-known for their control of hepatic glucose production and blood glucose levels. Insulin inhibited SeP transcription via the PI3K/Akt/FoxO1 $\alpha$  axis,<sup>(70)</sup> whereas the PGC-1 $\alpha$ -inducing glucocorticoid dexamethasone strongly enhanced SeP mRNA levels and protein secretion in cultured rat hepatocytes.<sup>(71)</sup> 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;<sup>(74)</sup> these earlier results can be explained by enhanced hepatic secretion of SeP induced by dexamethasone treatment.

The complex between FoxO1 $\alpha$  and its coactivator PGC-1 $\alpha$  is of crucial importance for transcriptional regulation of the gluconeogenic enzymes glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEP-CK).<sup>(27,72)</sup> Our observation that the selenium transporter SeP is regulated virtually like a gluconeogenic enzyme provides a rationale for the hypothesized link between selenium and carbohydrate metabolism.<sup>(71)</sup> Moreover, PGC-1 $\alpha$  is elevated in livers of animal diabetes models,<sup>(72)</sup> and has been demonstrated to promote insulin resistance.<sup>(28)</sup> A vicious circle is observed when diabetes is not treated accurately: high glucose up-regulates expression of PGC-1 $\alpha$  and gluconeogenic enzymes in the liver, resulting in overproduction of hepatic glucose and increased hyperglycemia.<sup>(72,75)</sup> We cultivated rat hepatocytes in the presence of high glucose (25 mM), and found an increase in SeP production paralleled by elevated PGC-1 $\alpha$  mRNA levels.<sup>(76)</sup>

Thus, elevated hepatic PGC-1 $\alpha$  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.<sup>(77,78)</sup> In parallel with gluconeogenesis, metformin attenuated hepatic

biosynthesis and secretion of SeP *in vitro*,<sup>(76)</sup> 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.<sup>(79)</sup>

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## Abbreviations

GPx	glutathione peroxidase
TrxR	thioredoxin reductase
NPC	Nutritional Prevention of Cancer
LDL	low-density lipoprotein
IRS	insulin receptor substrate
PTP	protein tyrosine phosphatase
FoxO	forkhead box class O
PGC	peroxisomal proliferator-activated receptor gamma coactivator
PI3K	phosphoinositide-3-kinase
ROS	reactive oxygen species
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
NOX	NAD(P)H oxidase
IR	insulin receptor
PIP3	phosphatidylinositol-3,4,5-triphosphate
SeP	selenoprotein P
HNF-4 $\alpha$	hepatocyte nuclear factor 4 $\alpha$
G6Pase	glucose-6-phosphatase
PEP-CK	phosphoenolpyruvate carboxykinase

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