The debate on selenium as risk factor for type 2 diabetes: Evidence for interplay of selenium and energy metabolism

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Abstract

The essential trace element selenium (Se) has a long track record for anti-diabetic and insulin-mimetic properties. By contrast, more recent epidemiological data have suggested potential pro-diabetic effects of supranutritional Se intake in humans. Animal and cell culture studies have provided evidence that dietary Se compounds and selenoproteins can affect both the pancreatic insulin secretion and the insulin sensitivity of target tissues, thereby interfering with insulin-regulated metabolic pathways. To gain insight into underlying molecular mechanisms, we investigated the transcriptional regulation of the major Se-containing plasma protein selenoprotein P by factors related to carbohydrate metabolism, and vice versa, the influence of Se compounds on the insulin signalling cascade. Liver-derived selenoprotein P (SeP) is crucial for Se homeostasis acting as plasma Se transporter. We identified high glucose and glucocorticoids as positive regulators of hepatic SeP biosynthesis, whereas insulin attenuated SeP expression and secretion. Thus, SeP is
regulated like a gluconeogenic enzyme by factors controlling the hepatic glucose factory under physiological and pathophysiological conditions. On the other hand, we showed that Se oversupply causes alterations in insulin-regulated energy metabolism in vitro and in vivo: Sodium selenite, a Se source in dietary supplements, delayed insulin-triggered phosphorylation of protein kinase B (Akt) and FoxO transcription factors and decreased glucose uptake in cultured myocytes. In a small pilot study, healthy male pigs were fed a supranutritional Se diet (0.5 mg Se as Se-enriched yeast/kg). After 16 weeks, fasting plasma concentrations of insulin, cholesterol and triacylglycerols were non-significantly elevated in the Se-supplemented animals, whereas fasting glucose concentrations remained unchanged. Several alterations in expression and/or phosphorylation of transcription factors and enzymes point to a shift to increased lipid turnover in adipose tissue and skeletal muscle, induced by supranutritional Se. Taken together, current epidemiological and mechanistic data suggest a more careful handling of dietary Se supplements, even though supranutritional Se intake is probably not sufficient to induce diabetes in healthy individuals.

Introduction

The essential trace selenium (Se) has received attention for a plethora of (assumed) beneficial effects on human health and its unique biochemistry [1, 2]. Se is a constituent of the 21st proteinogenic amino acid selenocysteine (Sec) that is co-translationally incorporated into 25 human selenoproteins [3]. Even though the selenoproteome is rather small, biosynthesis of selenoproteins has been shown to be essential for mammals: transgenic mice with a disrupted selenocysteine-tRNA<sup>Sec</sup> gene exhibit early embryonic lethality [4]. Humans, whose selenoprotein biosynthesis is impaired due to a rare heterozygous defect in the Sec insertion sequence-binding protein (SECIS) 2 gene, suffer from a multisystem disorder [5]. Many selenoproteins are enzymes with one Sec residue in their active center. Glutathione peroxidases (GPx) and thioredoxin reductases (TrxR) contribute to degradation of reactive oxygen species (ROS) and regulation of the cellular redox homeostasis [2]. Iodothyronine deiodinases (DIO) are
involved in the synthesis of thyroid hormones [6]. Seven selenoproteins are localized in the endoplasmic reticulum, where they contribute to the quality control of protein folding and the regulation of calcium homeostasis [7]. Selenoprotein P (SeP) contains up to 10 Sec residues and serves mainly as plasma Se transporter [8].

The biosynthesis of several key selenoproteins such as GPx1 and SeP decreases when Se supply is low [9,10]. Concentrations of the two major selenoproteins in plasma are used as biomarkers of Se status: Maximal GPx3 activity in plasma is achieved at a daily intake of ~70 µg Se [11], whereas SeP plasma levels reach a plateau at ~105 µg Se/day [12]. In Germany and in most other European countries, the average Se intake from the habitual diet (< 50 µg Se/day) is below those levels, but within the recommended range for adequate nutrition of humans (30 to 85 µg Se/day) [1, 13]. Compared to Europe, Se intake in the U.S. is much higher with 93 and 134 µg/day for males and females, respectively [1, 13]. Overt Se deficiency occurs very rarely in humans. Nevertheless, the consumption of Se-enriched dietary supplements is common in Europe and more so in the U.S., where one-third of the population regularly ingests multi-vitamin/mineral supplements [14]. Supplements can provide an additional 10-200 µg Se/day, in form of inorganic Se compounds such as sodium selenite and selenate as well as organic Se-compounds, e.g. Se-enriched yeast and garlic containing selenomethionine and gamma-glutamyl-Se-methylselenocysteine [1, 15].

Adequate and/or supranutritional Se intake has been proposed to be beneficial in terms of cancer prevention, dating from a landmark study in the 1970s that reported an inverse correlation of Se intake levels with cancer mortality among individuals from 27 countries [16]. Moreover, Se might be useful for protection against oxidative stress-related chronic diseases of the cardiovascular system and the brain and in the therapy of inflammatory disorders, viral diseases and sepsis [1, 2, 17]. There is increasing evidence that Se may delay the inflammatory process in autoimmune thyroid disease [18]: a recent study showed an improved quality of life and a slower progression of orbitopathy in patients with Graves’ disease (M. Basedow), who were supplemented with 200 µg selenite/day [19]. On the other hand, Se has a very narrow therapeutic window and a U-shaped dose-response curve. Cases of Se toxicity have been observed in
humans and animals consuming plants grown at Se-rich soil or after accidental ingestion of very high Se doses [1]. Currently, the “tolerable upper intake level” is set at 300-450 µg Se/day for adults [1, 13]. To assess the prospects of dietary Se supplementation for human health, it should also be taken into account that Se intake above nutritional requirements could trigger adverse side-effects even below toxic levels.

Epidemiological evidence in regard to selenium as risk factor for type 2 diabetes

The much discussed Nutritional Prevention of Cancer (NPC) trial has shown that there is probably no light without shadow when Se shall be used for dietary supplementation: In order to examine whether Se could suppress the recurrence of skin cancer, 1312 patients from the Eastern U.S. with a history of basal cell or squamous cell carcinomas of the skin received for 4.5 years either 200 µg Se/day in the form of Se-yeast or a placebo [20]. On the one hand, the NPC trial provided strong evidence for a tumor-preventive capacity of Se by revealing decreased overall cancer mortality and a lower incidence of prostate and colorectal cancer in Se-supplemented male subjects with relatively low (< 122 ng/mL baseline plasma Se) initial Se status [20, 21]. On the other hand, Se-supplemented NPC participants with high baseline plasma Se levels (> 122 ng/mL; top tertile) were more likely to develop type 2 diabetes mellitus (T2DM) than those assigned to placebo [22]. An ongoing discussion regarding the safety of dietary Se supplements has arisen from this unexpected finding.

In comparison to the NPC trial, the much larger selenium and vitamin E cancer prevention trial (SELECT) found that the risk to develop T2DM was slightly increased in the group of participants with daily administration of 200 µg L-selenomethionine. However, the increase in diabetes risk was non-significant [23], and this was confirmed by a recent follow-up of SELECT [24]. SELECT was carried out in healthy U.S. American men at the age ≥ 50 years, whose baseline plasma Se levels were even higher than in the participants of the NPC trial, ranging from 122 to 152 ng/mL [23]. According to two recently published small intervention studies, a short-term (6 weeks) dietary Se supplementation of healthy humans did not
induce T2DM or was even beneficial: 150 µg Se/day in the form of dairy-Se or Se-yeast did not cause an increase in fasting plasma glucose concentrations [25]. In comparison to the placebo group, HOMA-IR (homeostatic model assessment of insulin resistance) values were significantly lower in volunteers, who received 200 µg Se/day in the form of Se-yeast [26].

The majority (4 out of 6) of cross-sectional studies found a positive association between serum/plasma Se levels and T2DM. High serum/plasma Se levels were associated with increased fasting plasma glucose concentrations and/or increased total and LDL cholesterol concentrations in plasma [27-32]: Two of the four studies showing such positive associations were carried out in the U.S. (NHANES III and NHANES 2003-4) [27, 28], whereas the other two studies (SU.VI.MAX and Olivetti Heart Study) examined European populations [29, 30]. No significant associations were detected in the French EVA study and in a study from Singapore [31, 32]. In contrast, lower Se levels in toenails were reported among diabetic men with or without cardiovascular disease than among healthy participants of the U.S. American Health Professionals Follow-Up Study [33].

Longitudinal studies found no evidence for a diabetogenic role of Se in humans: a prospective analysis was undertaken to examine associations between serum Se concentrations and cardiometabolic risk factors in an 8-year follow-up of the Italian Olivetti Heart Study. However, baseline Se levels did not predict changes in plasma cholesterol concentrations between the baseline and follow-up examinations [30]. A 9-year follow-up of the French EVA study revealed a protective effect of high serum Se levels at baseline against the later occurrence of impaired fasting glucose that was specific for males [34].

Recently, we measured plasma adiponectin concentrations, a surrogate marker of insulin resistance and T2DM [35], in the elderly (60-74 years) participants of the UK Prevention of Cancer by Intervention with Selenium (PRECISE) trial. There was an inverse cross-sectional association between baseline plasma Se and adiponectin levels. However, Se supplementation for 6 months with 100, 200 or 300 µg Se/day in form of Se-yeast did not affect the adiponectin concentrations in plasma, arguing against Se-induced development of insulin resistance in this population [36].
Modulation of insulin secretion and signalling by selenium and selenoproteins

Insulin resistance and an impaired insulin secretory capacity due to progressive loss of pancreatic beta cell mass are hallmarks in the pathogenesis of type 2 diabetes mellitus. Supranutritional Se intake might contribute to the development of T2DM, as Se compounds and selenoproteins are capable of interfering with both insulin biosynthesis in the pancreas and insulin signalling in target tissues [37].

Expression and activity of glutathione peroxidases is particularly low in pancreatic islets, exhibiting only 5% of the values in liver [38]. Sodium selenite and selenate have been shown to stimulate biosynthesis and secretion of insulin in Min6 insulinoma cells and isolated rat islets \textit{in vitro}, probably by increasing GPx activity [39]. Paradoxically, an increase in GPx1 activity in pancreatic beta cells can elicit opposing metabolic outcomes \textit{in vivo} [40]. Beta cells of mice with global transgenic overexpression of GPx1 were hypertrophic, showing elevated insulin production and secretion. However, these alterations resulted in hyperinsulinemia, insulin resistance and obesity in aged animals [41]. In other animal models, increased GPx1 activity/expression had beneficial effects: Beta cell-specific GPx1 overexpression ameliorated hyperglycemia in \textit{db/db} mice and in streptozotocin-treated mice [42]. An adaptive increase in expression of antiapoptotic proteins and antioxidant enzymes including GPx1 has been proposed to contribute to survival of hypertrophic beta cells during chronic hyperglycemia in mice [43].

Earlier studies in the 1990s reported that high doses of the Se compounds sodium selenate and sodium selenite elicit insulin-mimetic effects in adipocytes and hepatocytes: 1 mM selenate stimulated glucose uptake in isolated rat adipocytes [44], and 10 µM selenite counter-acted glucagon-stimulated glycogen breakdown in the isolated perfused rat liver [45]. More recently, we compared the influence of four Se compounds (selenite, selenate, selenomethionine and methylseleninic acid) on insulin signalling and glucose uptake in skeletal muscle cells \textit{in vitro}: at a dose of 1 µM, selenite and methylseleninic acid delayed insulin-induced phosphorylation of protein kinase B (Akt) and forkhead box protein class O (FoxO)}
tion factors in L6 rat myotubes, whereas selenate and selenomethionine had no effect. Basal and insulin-stimulated glucose uptake in L6 myotubes was also attenuated by selenite and methylseleninic acid. In contrast, selenomethionine stimulated glucose uptake, but only at a high dose of 100 µM [46].

Patients with genetically impaired biosynthesis of selenoproteins exhibit enhanced systemic and cellular insulin sensitivity [5]. Two selenoproteins, GPx1 and selenoprotein P (SeP), have been reported to suppress the canonical insulin-induced signalling cascade in hepatocytes and myocytes [37]. GPx1 reduces hydrogen peroxide ($\text{H}_2\text{O}_2$) [9] that serves as second messenger to enhance early insulin signalling and to stimulate insulin-induced glucose uptake by transient inhibition of counter-regulatory phosphatases [47, 48]. As Se transport protein, SeP delivers Se for intracellular biosynthesis of GPx1 and other selenoproteins [8]. SeP has been shown to impair insulin signalling and to dys-regulate carbohydrate metabolism in hepatocytes and myocytes [49]. Knock-out of GPx1 in mice resulted in improved insulin-induced phosphorylation of Akt due to increased ROS generation and oxidation (inactivation) of the dual specificity protein phosphatase PTEN, and it protected the rodents from insulin resistance provoked by a high-fat diet [50]. Conversely, elevated GPx activity in the liver of sodium selenate-supplemented rats was associated with increased activity of protein tyrosine phosphatase 1B (PTP-1B) [51]. In addition to GPx1 and SeP, other selenoproteins such as selenoprotein S and methionine sulfoxide reductase have recently been proposed to be involved in the dys-regulation of carbohydrate metabolism induced by supranutritional Se intake [52].

We compared the expression of enzymes and transcription factors related to energy metabolism in major insulin target tissues of healthy male pigs fed either an adequate-Se (0.17 mg Se/kg) or a supranutritional-Se (0.5 mg Se/kg as Se-yeast) diet. After 16 weeks, fasting plasma concentrations of insulin, cholesterol and triacylglycerols were non-significantly increased in the Se-supplemented animals. Fasting glucose concentrations did not differ between the groups. We did not observe molecular alterations in the liver. In skeletal muscle of the supranutritional-Se pigs, pyruvate kinase was down-regulated, whereas the transcription factors FoxO1a and PGC-1α were up-regulated. In visceral fat of the supranutritional-Se pigs, mRNA
levels of the transcription factor SREBP1 were increased and basal phosphorylation of protein kinases (Akt, AMPK, MAPKs) was affected. This pattern suggests a shift to increased lipid turnover in adipose tissue and skeletal muscle of the Se-supplemented animals. However, supranutritional Se was not sufficient to induce type 2 diabetes mellitus [53].

Modulation of selenoprotein biosynthesis by factors related to energy metabolism

Alternatively, the observed cross-sectional associations between high plasma Se levels and hyperglycemia/dyslipidemia might arise from alterations in Se homeostasis and biosynthesis of selenoproteins as a side effect of a dys-regulated energy metabolism. Selenoprotein P contains around 60% of total Se in human plasma [54]. The vast majority of SeP circulating in plasma derives from the liver [8, 55]. Indeed, hepatic SeP biosynthesis has been shown to be increased under hyperglycaemic conditions: we reported that cultivation of isolated rat hepatocytes in the presence of high glucose concentrations (25 mmol/L vs. 11 mmol/L glucose) stimulated SeP mRNA expression and secretion [56]. A subsequent study corroborated and extended these findings by demonstrating up-regulated hepatic SeP expression by high glucose and palmitate as well as elevated SeP mRNA levels in the liver of animal T2DM models [49]. This might also explain observations of elevated SeP serum levels in individuals with prediabetes and T2DM [49, 57].

We found that the human SeP promoter contains a functional binding site for FoxO transcription factors [58]. FoxO1α and FoxO3 are involved in the control of the hepatic glucose factory by increasing the transcription of gluconeogenic enzymes through interaction with its co-activator PGC-1α and the transcription factor HNF-4α [59, 60]. We also identified a binding site for HNF-4α at the human SeP promoter, and we showed that SeP transcription is controlled by interaction of PGC-1α with FoxO1α and HNF-4α [61]. This explains both the observed down-regulation of SeP expression in hepatocytes by insulin and its up-regulation by glucocorticoids and high glucose [56, 58, 61]. Based on these results, we
developed the concept that SeP is regulated in hepatocytes like a gluconeogenic enzyme [37, 61]. In agreement with this idea, SeP mRNA levels in the liver of mice have been shown to be increased by fasting and to be decreased 1 h after feeding [49]. A recent study provided additional support by demonstrating that methylation of the involved transcription factors is required for transcription of both gluconeogenic enzymes and SeP [62].

Concluding remarks

The epidemiological evidence for a diabetogenic role of selenium in healthy humans is still rather weak and controversial, even though the majority of cross-sectional observations point to an association of high plasma/serum Se levels with biomarkers of type 2 diabetes mellitus such as hyperglycemia, dyslipidemia and low adiponectin plasma levels [27, 36, 37, 63-65]. Mechanistic studies have provided alternative explanations for the observed cross-sectional associations: dietary Se oversupply may affect pancreatic insulin secretion and insulin sensitivity of target tissues, probably through inducing abundant expression of selenoproteins. Conversely, hepatic biosynthesis of selenoprotein P, the major Se-containing protein in plasma, is increased under hyperglycaemic conditions.

Despite the induction of some alterations in energy metabolism, commonly applied selenium doses - as ingested through dietary supplements - are probably not sufficient to induce overt type 2 diabetes in healthy individuals. Nevertheless, it is recommended that individuals with high Se status should not ingest Se-containing supplements, as optimising, rather than maximising, exposure is the key to benefit most from Se while avoiding potential adverse effects [65].

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