Aus dem Institut für Tierernährung und Ernährungsphysiologie
der Justus-Liebig-Universität Giessen

Specific physiological features of inorganic selenium compounds regarding metabolism - in vivo and in vitro investigations with type II diabetic dbdb mice and healthy rats

Habilitationsschrift zur Erlangung des akademischen Grades
eines Dr. habil. und der venia legendi
verliehen durch den
Fachbereich 09 Agrarwissenschaften, Ökotrophologie und Umweltmanagement
der Justus-Liebig-Universität Giessen
für die Lehrgebiete
„Ernährungsphysiologie und Tierernährung“

vorgelegt von Dr. oec. troph. Andreas Müller,

wissenschaftlicher Assistent am

Institut für Tierernährung und Ernährungsphysiologie

I. Contents

II. Tables

III. Figures

1. Introduction and Problematic

Since its discovery by the Swedish chemist Jöns Jacob Berzelius in 1817 selenium (Se) has had a chequered history. Livestock disorders, commonly referred to as alkali disease or blind stagger were found to be endemic in areas with soils rich in Se. Similarly, the consequences of chronic Se intoxication in humans were noticed in seleniferous geographic areas long before Se was recognized as the causative agent. The prominent features of a Se intoxication are depression and fatigue, loss of hair and breakage of the nails. In brief Se was classified as poisonous and carcinogenic up to the late 1940s. The attitude to Se in life sciences began to change in the 1950s. In 1954 Pinsent observed that certain bacteria grew faster in Se-fortified media. In 1957, Schwarz and Foltz discovered that Se was contained in "factor 3", a still ill-defined compound isolated from hog kidney, which efficiently prevented the experimentally induced fatal liver necrosis of rats that were fed a diet based on torula yeast and sucrose. It soon became apparent that factor 3 could be replaced by a variety of inorganic or organic Se compounds in the liver necrosis model. Consequently Se was considered as acting as an essential trace element. In the 1960s this view was corroborated, since various syndromes such as white muscle disease in cattle, mulberry heart disease and hepatosis dietetica in pigs as well as exsudative diathesis in poultry could be attributed to insufficient Se supply. After identification of cellular glutathione peroxidase 1 as a Se containing protein the protective effect of Se regarding the above mentioned tissue-destructing disorders could soon be attributed to the peroxide reducing and antioxidant features of Se representing the catalytically active centre of glutathione peroxidase 1. The discovery of cellular glutathione peroxidase 1 was followed by the finding to date of six further tissue specific glutathione peroxidases with a peroxide reducing function. Cellular and mitochondrial thioredoxin reductase 1 and 2 as well as thioredoxin glutathione reductase, also termed thioredoxin reductase 3, represent another class of relatively small redox active selenoproteins which are expressed in a number of mammalian tissues. Thioredoxin reductases are involved in desoxyribonucleotide synthesis and in the reduction of a broad spectrum of other compounds not completely known yet. For instance ascorbate and selenite are compounds undergoing reduction by thioredoxin reductases. A further important class of functional selenoenzymes is represented by three members of the iodothyronine deiodinase family participating in the precise regulation of thyroid hormone metabolism. One of the remaining selenoproteins to which a main research focus is dedicated is selenoprotein P which plays a key role in the interorgan- and tissue-distribution of Se in mammals.

In recent years a number of studies were carried out investigating specific physiological properties of single Se compounds as well as additional properties of Se and functional selenoproteins on the expression of other genes. Examples for these specific physiological features however appear partly contradictory. For instance, on the one hand it could be

shown that the application of high supranutritional selenate doses (Se oxidation state +VI) mimicked insulin effects and featured strong antidiabetic and antiadipogenic properties whereas the overexpression of glutathione peroxidase 1, representing the best characterised antioxidant selenoprotein, has been demonstrated as promoting the development of obesity and insulin resistance in mice. Consequently the aim of the present studies was to examine the molecular mechanisms underlying the differentiated action of Se and in particular of inorganic Se compounds on metabolic processes critically.

2. Literature survey

2.1 Selenium metabolism in mammals

Selenium (Se) belongs to the chalcogens and many of its chemical properties (outer valence shell electronic configuration, atomic size, bond energy, ionisation potential, electronegativity) are similar to those of sulphur. As in the case of sulphur, Se occurs in the oxidation states –II (selenide), 0 (elemental selenium), +IV (selenite) and +VI (selenate). In its elemental oxidation state (0) Se forms red crystals with a hexagonal ring structure [1]. Besides these similarities there exist nevertheless some important differences between the two elements regarding the chemistry of their oxyanions and the acid strengths of their hydrides. Since Se compounds are preferentially reduced and sulphur compounds undergo oxidation, the following inorganic reaction between quadrivalent Se and quadrivalent sulphur displays the basis for physiological chemistry of Se in mammals:

+IV +IV ±0 +VI

$H_2SeO_3 + H_2SO_3 \rightarrow Se + 2\ H_2SO_4 + H_2O$ [2, 3].

A translation of this inorganic reaction to the seleno- and sulphur-hydrides as present in the glutathione peroxidase reaction reflects a similar reaction pattern [4].

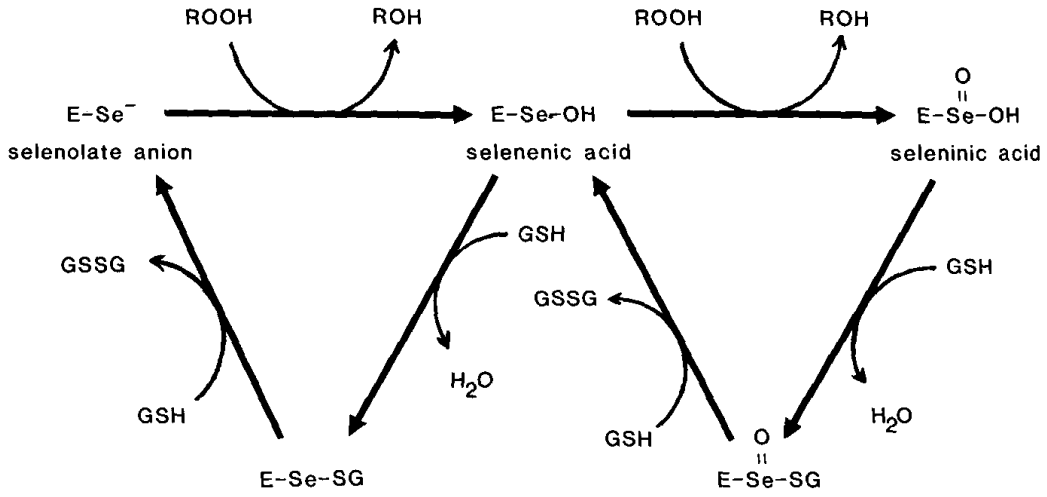


Figure 1: Physiological chemistry of Se and sulphur in glutathione peroxidase according to [4]

In the glutathione peroxidase reaction the sulphur compound glutathione undergoes oxidation to the disulfide, whereas Se is reduced.
At a physiological pH value Se in glutathione peroxidases is present as a selenolate anion. The reduction of a peroxide effects the oxidation to the selenol oxidation state (selenenic acid). Starting from the selenol oxidation state Se is reduced again in a two step reduction by glutathione (sulphur compound)

When taken up at the recommended level (animals: 0.15 – 0.30 mg Se/kg dietary dry matter, humans: 50 – 100 µg Se daily) [5, 6, 7, 8, 9] Se performs its physiological functions in the

body of animals and humans in form of a catalytically active selenocysteine residue in functional selenoproteins (for details see 2.2).
In human food Se is present in two major forms. Foodstuffs derived from animal sources mainly contain Se in the form of selenocysteine from functional selenoproteins, while Se from plant derived foodstuffs is present predominantly as selenomethionine. In mineral and trace element supplements Se is frequently added in the form of the inorganic salts sodium selenite (Se oxidation state +IV) and sodium selenate +VI) [10, 11].

The following information on Se metabolism is summarized in Figure 2.

Se from various dietary sources is absorbed by individual mechanisms in the small intestine of mammals. The amino acid derivatives selenomethionine and selenocysteine use the same carriers as their sulphur analogues methionine and cysteine [12]. Selenate uses a sodium-sulphate cotransporter for its absorption, which is driven by the activity of Na+/K+-ATPase at the basolateral enterocyte membrane [13]. In the lumen of the small intestine selenite partially reacts with glutathione or other thiols to selenotrisulfides, which are presumably taken up into the enterocytes by amino acid transporters. Another part of selenite diffuses through the apical membrane and reacts with thiols in the cytosol of enterocytes. The Se compounds mentioned above are absorbed to a high extent (> 85%) from dietary sources, but differences exist in the absorption time. As a result of the upstream selenotrisulfide synthesis selenite absorption is slower than selenate and selenomethionine absorption [12, 13]. Subsequently the selenocompounds are liberated into the blood stream at the basolateral enterocyte membrane and distributed to the various peripheral tissues. The exact transport mechanism for the various Se compounds is not fully understood yet. Selenomethionine associates with hemoglobin, while selenate and the remaining free selenite were found to be transported with α- and γ-globulins [14, 15, 16]. Orally administered selenite presumably enters the peripheral organs in the form of selenotrisulfides or is already reduced in the erythrocytes to the selenide oxidation state -II. Selenate is metabolized during and after its unmodified uptake by the peripheral tissues. This hypothesis of a distinctly different cellular metabolism for selenite and selenate is supported by an investigation into intermediary Se metabolites after intravenous injection of rats with both compounds [17]. Selenite was rapidly taken up by red blood cells, reduced in the erythrocytes to the selenide oxidation state –II and delivered to peripheral organs (liver) in an albumin bound form. In contrast unmodified selenate (Se+VI) could be detected in the bloodstream and in peripheral organs. Thus it can be assumed that the successive selenate reduction to the oxidation state –II takes place after its uptake into peripheral organs [18, 19, 20]. A surplus of inorganic Se is stored in peripheral organs as “acid labile Se”. This Se fraction consists of Se bound

unspecifically to proteins (presumably via the formation of Se-S bonds) [21, 22]. The main excretion products of Se detected in urine are the methylated metabolites monomethylselenol (MMS) and trimethylselenonium (TMS). Methylated Se metabolites are formed from Se reduced to the oxidation state –II as well as from Se stored unspecifically in proteins as selenomethionine and from acid labile Se [23]. Se exhalation as dimethylselenide only takes place when Se is ingested in toxic doses.

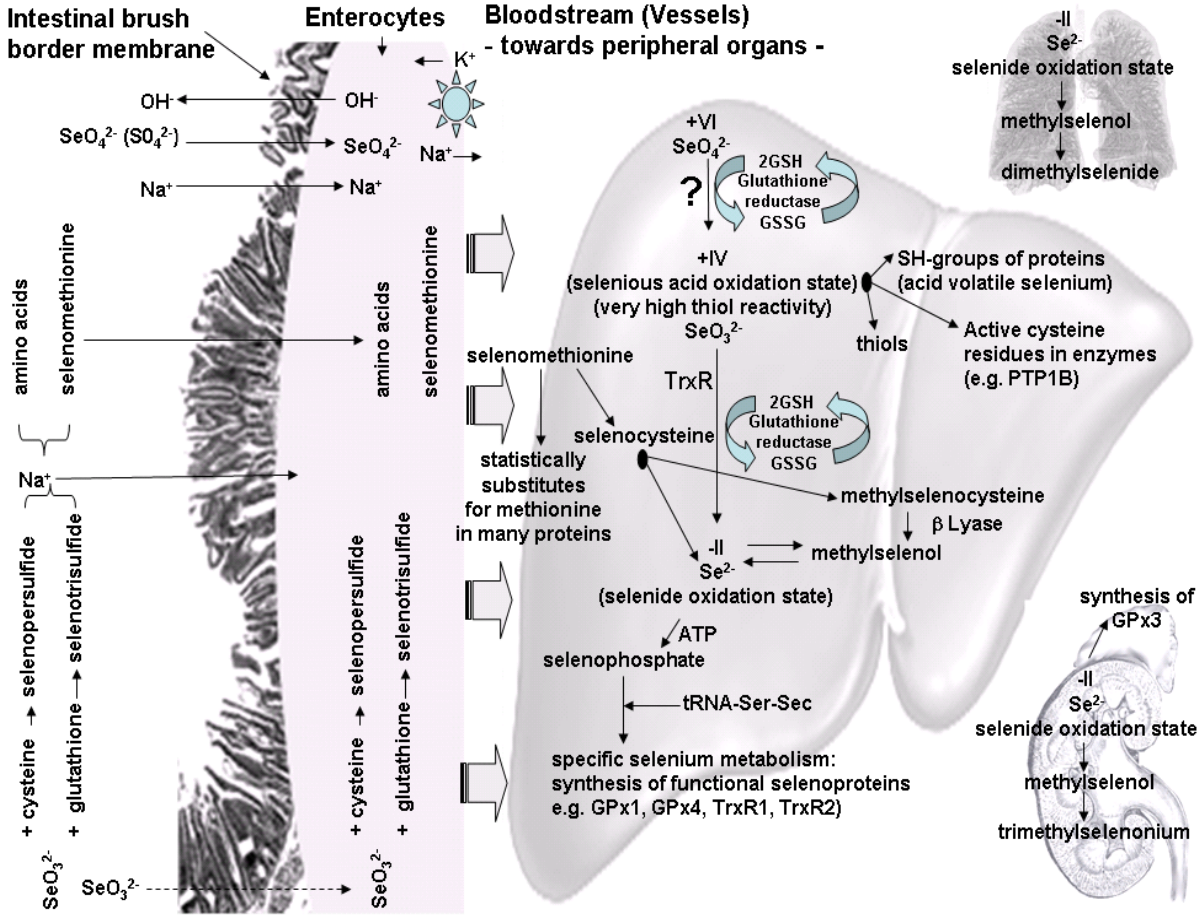


Figure 2: Current comprehension of mammalian Se metabolism [modified from 24,25, 256, 257]

- detailed information on the single pathways is given in the text of chapters 2.1 and 2.2 -

Selenomethionine represents the only Se compound which can be incorporated unspecifically into proteins instead of its sulphur analogue methionine. Thus selenomethionine containing proteins are termed non-specific Se containing proteins [24].

2.2 Functional selenoproteins – their role and regulation in mammals

Se metabolism of functional selenoproteins is also termed specific Se metabolism and it starts from Se derived from nutritional sources as well as from Se stored unspecifically as

selenomethionine in other proteins or from Se stored in the acid labile Se pool. For the synthesis of functional selenoproteins which are characterized by a catalytically active selenocysteine moiety it is a must to have Se from the above mentioned sources reduced to the selenide oxidation state –II [Figure 2]. During the further course of functional selenoprotein synthesis reduced Se is activated in an ATP-dependent reaction to selenophosphate by selenophosphate synthetase followed by the cotranslational synthesis of selenocysteine starting from a specific serine-tRNA-population and from the activated Se. Selenocysteine thereby is encoded by an unusal UGA-stop code in the mRNA of functional selenoproteins. The regulation of these complex processes including the cotranslational synthesis of selenocysteine and the incorporation of selenocysteine into the growing protein chain is controlled by the so-called SECIS element (selenocysteine insertion sequence) located in the 3´-untranslated mRNA region of functional selenoproteins. The knowledge that selenocysteine firstly is encoded by a base triplet and that it secondly represents a self-contained proteinogenic amino acid led to the establishment of selenocysteine as the "21st proteinogenic amino acid" [26, 27, 28, 29, 30].

As many as 26 functional selenoproteins have so far been identified in mammals. Much less is known regarding the exact functions fulfilled by these proteins. To date the highest level of knowledge exists regarding 3 main families of functional selenoproteins, namely glutathione peroxidases (GPxs), thioredoxin reductases (TrxRs) and iodothyronine deiodinases (DIOs). In the following some important facts are given on these enzyme families. Information on the the remaining selenoproteins, known so far, is displayed in Table 1.

2.2.1 The glutathione peroxidases 1 – 7 (GPx1 – GPx7)

Glutathione peroxidase GPx (EC 1.11.1.9 = GPx1) (EC 1.11.1.12 =GPx4) was the first specific mammalian selenoprotein identified [31, 32] and has received ever increasing attention. Today the family of glutathione peroxidases includes seven isoenzymes in mammals. One of the last additions to the list, GPx6, was identified in 2003 – 20 years after the discovery of GPx1 [32,33]. The black sheep of the family are GPx5 and GPx7, which are not selenoenzymes [34, 35]. The selenocysteine residue in GPx5 and GPx7 is replaced by a simple cysteine.

Glutathione peroxidases reduce and thereby detoxify different types of peroxides to their respective alcohols typically at the expense of glutathione according to the following reaction equation:

R-OOH + 2 GSH → R-OH + H_2O + GSSG [c.f. also Figure 1]. Apparently all of them share the same catalytic mechanism involving a strictly conserved catalytically active centre formed by selenocysteine, tryptophan and glutamine [4, 36, 37]. Glutathione peroxidases play an important role in the tissue's antioxidant defense.

Glutathione peroxidase 1

GPx1, which was later found to have selenoprotein properties, was originally discovered in 1957 [38]. It is an ubiquitous homotetrameric cytosolic enzyme (therefore often referred to as cGPx). GPx1 is abundant in the liver, in kidneys and in erythrocytes. Its concentration and activity are keenly dependent on the nutritional Se status [37, 39]. GPx1 is subject to a severe loss of its mRNA concentration and enzyme activity due to a lack of dietary Se supply. It therefore ranks low in the hierarchy of the functional selenoproteins [37]. Nevertheless GPx1 is one of the most important antioxidant enzymes, and besides the microsomal enzyme catalase it is responsible for H_2O_2 detoxification in tissues [40] according to the common reaction, displayed in Figure 1. Under conditions of a regular Se supplementation GPx1 accounts for the prevention of lipid- and protein-oxidation in cells. GPx1 knockout mice however show no obvious phenotype under normal conditions, yet when challenged with oxidative stress, significant pathologies become evident [41, 42, 43, 44, 45].

Glutathione peroxidase 2

GPx2 is found in the liver and in the gastrointestinal tract but the enzyme is absent in heart and kidney. Therefore GPx2 is often referred to as GI-GPx. Its distribution varies in the intestine and shows a decline from the crypts to the luminal surface [46]. GPx2 is a homotetrameric cytoplasmatic enzyme accepting a broad range of organic hydroperoxides such as t-butylhydroperoxide, linolic acid hydroperoxides and cumene hydroperoxide as its substrates. The enzyme does not reduce phosphatidyl choline hydroperoxide. GPx2 is conserved for a very long time under conditions of inadequate dietary Se supply, and therefore it ranks high in the hierarchy of functional selenoproteins [47, 48]. Some authors assume that GPx2 represents the first line of defense against organic hydroperoxides derived from food [37, 48, 49]. Regulatory functions for GPx2 are suggested as well, and the enzyme seems to participate in the regulation of apoptosis and proliferation [46]. GPx2 knockout mice do not have a unique phenotype. However, in GPx1-GPx2 double knockout mice inflammatory bowel disease and bacteria-induced tumors were observed [50].

Glutathione peroxidase 3

GPx3 is located extracellularly in the plasma, hence the acronym pGPx, and in the intestine [51, 52]. After selenoprotein P, GPx3 makes the second highest contribution to plasma Se concentration. The exact physiological function of this homotetrameric glycoprotein has not so far been exhaustively explained [53]. Furthermore there is no convincing evidence about the redox substrates used by GPx3 due to a nearly complete lack of glutathione in plasma. Presumably GPx3 uses glutaredoxin and thioredoxin for hydroperoxide reduction [53]. GPx3

acts as an efficient reductant towards hydroperoxides and presumably as an antioxidant towards proteins [54]. Furthermore it is speculated that GPx3 may have further regulatory functions. GPx3 expression is induced by hypoxia, and its deficiency seems to correlate with cardiovascular events and cancer [55, 56]. Like GPx1 Se deficiency leads to a fast and strong decrease in GPx3. Therefore GPx3 ranks low in the hierarchy of glutathione peroxidases. GPx3 is primarily expressed and synthesized in the renal proximal tubules. Subsequently the enzyme is liberated into plasma [57].

Glutathione peroxidase 4

In contrast to the glutathione peroxidases 1-3, GPx4 (EC 1.11.1.12) is a monomeric enzyme with a number of unusual features. By using alternative initiation sites (Met1 or Met28), mitochondrial and cytoplasmatic isoforms of GPx4 are generated during its synthesis. Moreover GPx4 uses the broadest substrate range of all glutathione peroxidases. GPx4 is even able to reduce phospholipid hydroperoxides and therefore is often referred to as PH-GPx. The enzyme is even capable of reducing hydroperoxides still integrated in cell membranes and it may thus play a role as a universal antioxidant in the protection of biomembranes [58, 59]. GPx4 is additionally involved in redox signalling and regulatory processes, such as inhibiting lipoxygenases and apoptosis [37, 60]. In the testes, where it accounts for almost the total Se content GPx4 transforms into a relevant structural protein of the sperm's midpiece [61, 62, 63]. GPx4 is therefore required for sperm motility and fertility rather than for antioxidant defense of spermatozoa [63]. Thus it is not surprising that a long term Se deficiency and possibly GPx4 polymorphisms are associated with male infertility [61, 65, 66]. GPx4 knockouts appear multimorbid and die at an early embryonic stage. The causes for retardation and the early death of GPx4 knockouts seem to be very complex and require further investigation [67]. Similar to GPx2, expression and activity of GPx4 are maintained for a long time during alimentary Se deficiency. Therefore GPx4 ranks very high in the hierarchy of glutathione peroxidases [37].

Glutathione peroxidase 6

GPx6 was discovered using an in silico approach. So far, GPx6 expression, as judged by its mRNA concentration, is present only in olfactory epithelium and embryonic tissues [33]. GPx6 is expressed in or near the Bowman's glands which is a site for several olfactory-specific biotransformation processes. This finding solely suggests but does not prove yet a function for GPx6 in olfaction.

The following two glutathione peroxidases, namely GPx5 and GPx7, are non-selenocysteine containing GPx isoforms and therefore the text regarding these enzymes is italicised.

However, both enzymes share the common reaction mechanism postulated for the classical glutathione peroxidases 1, 2, 3, 4 and 6. In the case of GPx5 the non-selenoenzyme presumably substitutes partially for its selenocysteine containing relative GPx4. Therefore some basic information is also provided for these two glutathione peroxidases.

Glutathione peroxidase 5

GPx5 is a non-selenocysteine containing glutathione peroxidase isoform and found exclusively in the epididymis [34]. It exists as a secretion protein as well as a membrane bound enzyme. It was suggested that GPx5 functions as a backup for the selenocysteine-containing isoforms of GPx4 in sperm [68]. The expression level of GPx5 in mammals is however very low and further research is needed to determine the relevance and the exact functions of the enzyme [69].

Glutathione peroxidase 7

GPx7 - like GPx5 – is a non-selenocysteine-containing glutathione peroxidase isoform. GPx7 has little detectable glutathione peroxidase activity in vitro [35]. GPx7 is reported as one protective factor against breast cancer by its antioxidant function towards oxidative stress deriving from the metabolism of polyunsaturated fatty acids [35].

2.2.2 The cellular and mitochondrial thioredoxin reductases 1 and 2 (TrxR 1 and 2) and thioredoxin glutathione reductase = (TrxR3)

The classical thioredoxin system is formed by thioredoxin reductase (TrxR; EC 1.8.1.9) catalysing the following reaction using its associated substrate thioredoxin (Trx) at the expense of NADPH:

$TrxS_2$ + NADPH + H^+ $\longrightarrow$ $Trx(SH)_2$ + $NADP^+$. Reduced thioredoxin serves as a reducing equivalent for various target molecules such as ribonucleotide reductase [70]. Thioredoxin reductases belong to a family of homodimeric pyridine nucleotide-disulfide oxidoreductases, inclusive of lipoamide dehydrogenase, glutathione reductase, and mercuric ion reductase [71]. Two very distinct classes of thioredoxin reductases have evolved:

1. small thioredoxin reductases (subunit Mr ~35 kDa) which are present in prokaryotes and fungi
2. large thioredoxin reductases (subunit Mr approx. 55 kDa) which are present in higher eukaryotes and mammals.

Historically these two classes were considered to be mutually exclusive until a report on the thioredoxin system in the green algae Chlamydomonas reinhardtii was published [72]. This organism is unusual since it harbours both classes of thioredoxin reductases. However, due

to the fact that small TrxRs are absent in the human genome, they will not be further elucidated in this section. That mammalian cellular thioredoxin reductase is a selenoprotein was first discovered in human carcinoma cells [73]. This result could soon be confirmed for the other two mammalian thioredoxin reductases 2 and 3 [74, 75]. The selenocysteine residue of thioredoxin reductases was thereby identified uniquely as the penultimate amino acid [76]. A relatively large number of thioredoxin reductase splice variants exists and may be relevant for regulating organelle- and cell-specific localization [77, 78]. Interestingly, knockout experiments that eliminated the two known thioredoxin reductase isoforms 1 and 2 are both lethal in utero [79, 80]. The thioredoxin system is involved in a myriad of cellular and intercellular processes, and today it is difficult to distinguish the most important pathways. It should be noted that thioredoxin reductases exhibit an unusually broad substrate spectrum, ranging from low molecular weight compounds to large proteins [82].

Thioredoxin reductase 1

TrxR1 is an ubiquitous cytoplasmatic housekeeping enzyme. It is involved in many aspects of cellular redox regulation [83]. It is capable of inducing apoptosis if the enzyme does not contain selenocysteine or if this residue is blocked, e. g. by chemotherapeutic agents [84]. Besides its primarily important involvement in desoxyribonucleotide synthesis, these findings predestine TrxR1 as a very interesting target for chemotherapy [85]. TrxR1 is also secreted into plasma but the importance of this finding could not be explained as yet [86].

Thioredoxin reductase 2

TrxR2 is located in mitochondria with the highest levels in the prostate, testes, liver, uterus and small intestine and intermediate levels in brain, skeletal muscle, heart and spleen [87]. Two splice variants designated SelZf1 and SelZf2 are described at the mRNA level [88]. However, these isoforms lack the N-terminal redox active site CVNVGC and remain catalytically inactive. Whether these isoforms are artefacts or have another function has not yet been established. TrxR2 knockout studies led to early embryonic death with signs of severe anaemia, apoptosis in the liver and heart abnormalities. A heart-specific knockout causes a dilatative cardiomyopathy and early death, similar to Keshan disease [89, 90]. Both TrxR1 and TrxR2 are moreover capable of reducing a broad range of other substrates, e.g. dehydroascorbate, selenite, and proteins with oxidized SH-residues [91, 92].

Thioredoxin reductase 3 = (Thioredoxin glutathione reductase)

TrxR3 is a testis-specific enzyme. Unlike TrxR1 and TrxR2 it can reduce glutathione disulfide since it contains a N-terminal 1-Cys glutaredoxin-like domain. It is located in the endoplasmatic reticulum [93]. Specific functions of TrxR3 are however unknown so far.

To date only little information exists on thioredoxin reductase regulation by dietary Se and/or other nutrients. From the few studies dealing with TrxR expression due to dietary Se manipulation it is evident that thioredoxin reductase expression and activity remain almost stable during a short term Se deficiency of up to 10 weeks. Therefore it can be assumed that thioredoxin reductases, comparable to GPx2 and GPx4, rank high in the hierarchy of functional selenoproteins [81, 82].

2.2.3 The iodothyronine deiodinases 1 – 3 (DIO 1 – 3)

The first deiodinase (DIO) identified as a selenoenzyme was DIO1 in 1990 [94, 95]. DIO1 was amongst the first mammalian selenoproteins discovered. Deiodinases cleave specific iodine carbon bonds in thyroid hormones [Figure 3], thereby regulating their hormonal activity. Thyroid hormones and in particular T4 (= 3,3´,5,5´-tetraiodo-L-thyronine, half-life period: = 7 days), T3 (= 3,3´,5-triiodo-L-thyronine, half-life period: 1 day) and reverse T3 (= 3,3´,5´-triiodo-L-thyronine) are of crucial importance to human health as they regulate a number of metabolic functions. Thyroid hormones act primarily via intracellular receptors as transcription factors and are required for normal growth and development, for thermogenesis, and for the regulation of basal metabolic rate. Normal thyroid function depends on the two trace elements iodine and Se. The thyroid gland has the highest Se content per gram among all organs [96]. Thereby Se is not only present in the deiodinases, but also in glutathione peroxidases which are presumably required for the peroxide-dependent formation of T4. Iodine seems to be solely used for thyroid hormone synthesis which makes the thyroid system particularly vulnerable to iodine deficiency [97]. Diseases such as myxedematous cretinism and Kashin-Beck may result from combined iodine-Se-deficiencies [98, 99]. Today three types of deiodinases are known which do not only differ in sequence and structure, but they also catalyze different reactions. However, most enzymatic deiodination reactions require an endogenous reductant that has not yet been identified for the deiodinases. In fact, it is suggested that deiodinases may act as “single-use enzymes” *in vivo* [100]. The thyroid hormone system is very complex, especially in the anterior pituitary of the brain, which releases the thyroid-stimulating hormone; but different peripheral tissues must also respond appropriately to circulating T3 and T4.

Deiodinase 1

DIO1 (EC 1.97.1.10, formerly 3.8.1.4) was identified in 1990 as a selenoenzyme by two groups independently [94, 95]. The Sec-encoding UGA was discovered a little later [101, 102]. DIO1 is a homodimeric plasma membrane protein and primarily deiodinates the 5’-position of the phenolic ring [Figure 3], but it can also deiodinate the 5-position under certain circumstances. By its 5’-deiodination activity DIO1 converts L-thyroxine (T4), which is

secreted by the thyroid gland to the highest extent, to T3 representing the actual active thyroid hormone. Furthermore almost inactive reverse T3 (rT3) can be converted to 3,3´-diiodo-L-thyronine. DIO1 expression is high in the liver, kidney, thyroid and pituitary gland. Trace levels of the enzyme can be detected in most other tissues with the exception of the brain, where DIO2 predominates. The relative contribution of different tissues to plasma T3 levels via DIO1 activity is difficult to assess. Fast-exchanging tissues, such as liver and kidney, however appear to represent the primary sources. More than 80% of T4 is converted to T3 outside the thyroid. Moreover conversion to T2 and T1 is almost exclusively done outside the thyroid gland [103]. DIO1 expression is induced by elevated T4 and T3 levels and responds to increased carbohydrate intake. 6-propyl-2-thio-uracil (PTU) [104] and gold-I-complexes, such as aurothioglucose, were described as potential inhibitors of several selenoenzymes including DIO1 [101, 105, 106].

Deiodinase 2

Establishing DIO2 (EC 1.97.1.10) as a mammalian selenoenzyme was under debate until it could be proven that the functional SECIS element was present in the mRNA of the enzyme, even though it was located unusually far away (5.4 kb) from the UGA codon [107]. DIO2 is an ER-membrane protein [108]. It deiodinates the 5'-position with a preference for T4 over rT3. DIO2 is present in the central nervous system, in the pituitary gland and in the thyroid gland as well as in skeletal muscle, heart muscle, in the placenta and in brown adipose tissue. Low levels are detectable in the kidney and in the pancreas. As the predominant DIO form in the brain, DIO2 is responsible for more than 75 % of the local T3 production in this organ. T3 production within the brain is necessary, as there is only a minimal absorption of T3 from the blood stream across the blood-brain barrier [109]. Interestingly the T4:T3 ratio 1 in the brain is approximately 1:1 in comparison to other tissues in which T4 is more abundant. Total T3 produced in peripheral tissues provides ~50 % of total plasma T3. Unlike DIO1, DIO2 is down-regulated by both, increasing T4 and rT3 levels and rapidly degraded via an ubiquitin-dependent pathway (half life: minutes to 1 h) [110]. All these mentioned facts allow a rapid fine tuning of local T3 production in response to changes in circulating T4 levels. With the exception of mild growth retardation and hearing loss DIO2 knockout mice only show little gross phenotype abnormalities [111, 112]. DIO2 activity is only minimally affected by PTU and aurothioglucose.

Deiodinase 3

DIO3 (EC 1.97.1.11) was discovered in 1995 [113]. Unlike DIO2, DIO3 deiodinates the 5-position of the tyrosyl ring [Figure 2]. The resulting products cannot bind to the nuclear T3 receptor and have therefore no classical thyromimetic effect. Thus, the prime physiological

function attributed to DIO3 is the inactivation of T3 and T4. Brain, placenta and pregnant uterus express considerably high amounts of DIO3. Persistently high levels of DIO3 and low levels of T3 may however have deleterious effects upon central nervous system development and brain function [113]. The particular expression pattern for DIO3 presumably reflects the organism's attempt to protect the fetal central nervous system from inappropriate levels of T4 and T3 [114]. DIO3 is induced with increasing T4 levels, and like DIO2 the enzyme is almost insensitive towards PTU and gold(I).

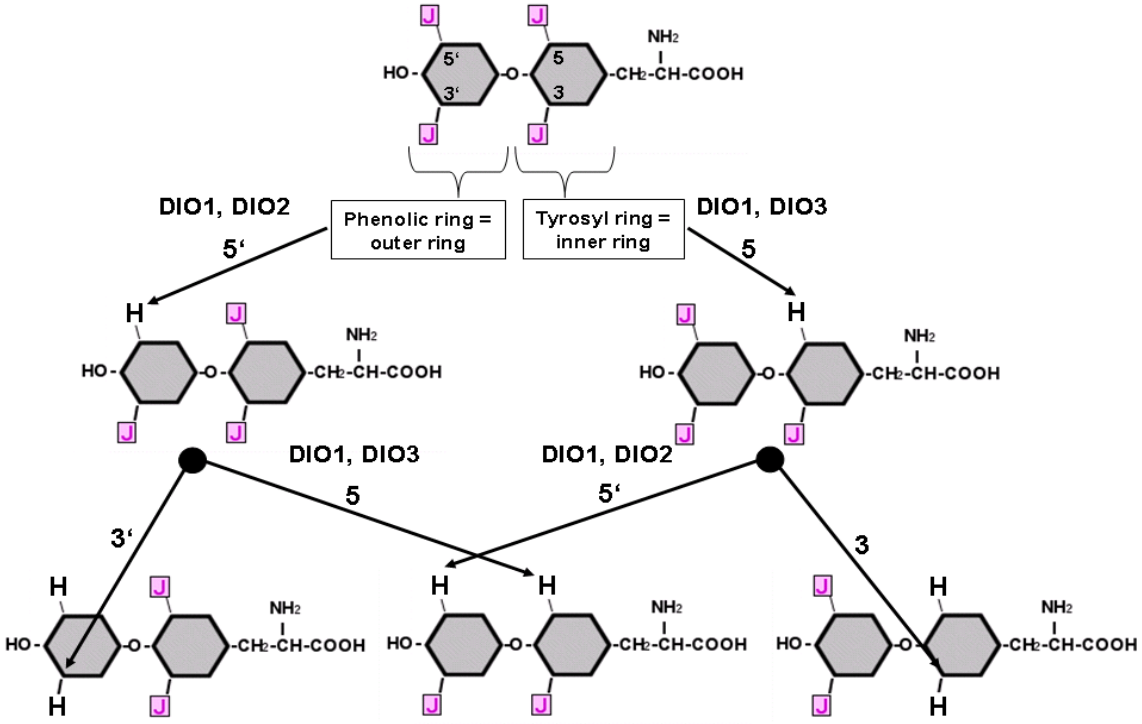


Figure 3: Metabolic pathways of L-thyroxine

Shown are the structures of L-thyroxine (T4), its primary metabolites T3 and reverse T3 and their metabolic fates. The respective deiodinases are indicated. Alternative pathways, such as glucuronidation or sulfation, are not shown.

2.2.4 Selenoprotein P

Selenoprotein P not only represents the major selenoprotein in plasma, but also provides more than 50 % of total plasma Se [115]. Its existence was originally discovered in 1982 in rats and later confirmed for other mammalian species [116, 117]. SelP mRNA is detectable in a number of tissues. The majority of SelP occurring in plasma (>80 %) is secreted by the liver and presumably enters target cells via a receptor-mediated mechanism [118, 119]. Unlike most selenoproteins that contain only one selenocysteine per polypeptide chain mammalian SelP contains up to 10 selenocyteine residues per chain [120–122]. Two selenocysteines apparently form selenyl-sulfide bridges with cysteine residues [123]. SelP is

an established marker for the nutritional Se status [118, 124]. Its extracellular localization and the repression of SelP expression during acute phase reaction, as well as its intrinsic high Se content and plasma concentration, led researchers to the assumption that the primary functions of SelP consist in the storage and the transport of Se [116, 125, 126, 127]. Studies using radiolabelled ^{75}Se support this hypothesis by demonstrating Se enrichment in brain, kidney and testes [116, 128]. The tendency of Se to bind heavy metal ions and its redox properties also suggest functions for SelP as a plasma antioxidant and as a heavy metal antidote [129–133]. However, the belief that SelP functions as an effective antioxidant is challenged by the fact that no efficient reductant has been identified in sufficient concentrations in the plasma as yet [134, 135]. In 2003, more than 20 years after its discovery, results regarding the consequences of SelP-gene disruption were published for mice [136-138]. These results show that SelP functions as a Se-transport-molecule, distributing nutritional Se from the liver to peripheral organs. SelP-knockout mice had an 80–90 % decreased plasma Se concentration. Se tissue concentrations and selenoenzyme activities dropped markedly in the brain, in kidney, and in testes [138]. Whereas mice with a complete tRNA [Ser]Sec knockout were not viable in utero, embryonal development in SelP knockouts showed no obvious deficits. Symptoms which could be attributed to the SelP knockout did not become evident prior to the third postnatal week [139]. These data show that SelP itself is not of vital importance during early development, and they further indicate that SelP is not the underlying cause of embryonal lethality in selenoprotein tRNA [Ser]Sec knockout mice. Deficits of a SelP knockout, occurring after some weeks of life include a reduced weight gain, sporadic fatalities and cerebral symptoms such as ataxia [140]. Interestingly, most symptoms, including the cerebral signs can be avoided by high supranutritional Se supply (studied for selenite) or by Se transfer from the females to their offspring by suckling [138, 140]. The symptomatic recovery correlates with increasing selenoenzyme activities in the affected tissues. The only symptom not responding to supranutritional dietary Se supplementation in SelP-knockout mice is the reduced fertility in males. Testicular Se levels and enzyme activities remain low [138, 141]. A withdrawal of supranutritional Se again leads to a rapid loss of Se in all organs, including the brain, and consecutively clinical symptoms (re)develop [118]. In a further study hepatic SelP release was selectively prevented by a liver-specific tRNA[Ser]Sec knockout [118]. As a consequence SelP levels in plasma dropped markedly. However, unlike in the complete SelP knockout experiments, neither a decrease in cerebral Se concentration and cerebral selenoprotein levels, nor clinical neurological symptoms could be observed in the liver knockout model [118, 138]. Only renal Se concentration and the secretion of GPx3 from the kidneys were diminished. These data indicate that SelP is required in the brain to retain Se and that transport mechanisms other than hepatic SelP exist to provide Se for most organs

at a supranutritional supply. In summary these results give evidence for the current hypothesis that Se from nutritional sources reaches the liver in a first-pass effect. In the liver Se is used partially for the synthesis of SelP which again functions as intermediary Se storage [118, 142]. In contrast to most other low molecular weight Se compounds (selenotrisulfides and acid labile Se) SelP is rather inert. SelP is then secreted into plasma and delivered to target tissues where it is taken up via a receptor-mediated mechanism. Within the cell SelP and subsequently selenocysteine are degraded to liberate Se which again is recycled for the synthesis of novel selenoproteins. Decreased SelP mRNA levels often occur in prostate cancer and suggest that SelP expression is down-regulated in this cancer type [143]. Indeed, Se levels are commonly reduced in the plasma of prostate cancer patients. Contrary to this Se concentration is even lower in patients with benign prostate hyperplasia [144]. Furthermore tissue Se concentrations have also been reported to be increased in prostate cancer [145].

2.2.5 Information on further functional selenoproteins

Information on further functional selenoproteins, known to date, is summarized briefly in Table 1.

Table 1: Comprehensive description of further functional selenoproteins and their functions as known to date

Functional selenoprotein	Available information on structure, functions, catalyzed reactions and subcellular localization	Tissue localization	Reference(s)
Selenophosphate synthetase (Sps2, selD2)	•Provides selenophosphate during the synthesis of functional selenoproteins: $HSe^- + ATP + H_2O \rightarrow HSePO_3^{2-} + HPO_4^{2-} + AMP$	all mammalian tissues	146 - 151
PES (prostate-epithelial-specific-selenoprotein)	●300 kDa holoenzyme with 15 kDa subunits ●Antioxidant function not yet proved	prostate	152
SeP 15	●H_2O_2 degradation ●Associated with UDP-glucose-glycoprotein-glucosyl-transferase in the endoplasmatic reticulum and therefore involved in quality control of miss-folded proteins	prostate	153 - 158
SeP 18	●No information available	liver, spleen, kidney, brain	159
SSPs (small selenoproteins)	●Different SSPs with molecular weights of 3, 4, 5, and 7 kDa were described ●Their function remains unknown as yet	adrenals, brain, epididymis, pituitary, thyroid, prostate	160
SelH	●Globular protein with 122 amino acid residues. The Sec-residue is at position 44 ●Presumably possesses a redox function by forming a selenyl-sulfide bridge with Cys-40	liver, spleen, kidney, brain	161
SelI	●Structural data are unavailable as yet in silico sequence analysis predicts up to 10 transmembrane domains ●Biochemical functions unknown so far	liver, spleen, kidney, brain	161
SelK	●Structural data are unavailable as yet ●Membrane associated protein ●Biochemical functions unknown so far	originally cloned in hemopoietic stem cells	161, 162
SelM	●Protein of the endoplasmatic reticulum (ER), containing presumably 122 amino acid residues, of which the first 23 form an ER signal sequence ●A CXXU motif indicates a redox function	many tissues with the highest level in brain and lower levels in liver and spleen	161, 163
SelN	●Glycoprotein retained in the ER ●Some rare myopathies (e.g. multiminicore myopathy, desmin-related myopathy with Mallory anti-bodies) are referred to as SelN deficiency	skeletal muscle, brain, lung	161, 164 - 167
SelO	●With 669 residues and a molecular weight of about 73 kDa a large protein ●The C-terminal CysXXSec motif indicates redox properties	no information available as yet	161

Table 1 (continuation): Comprehensive description of further functional selenoproteins and their functions as known to date

Functional selenoprotein	Available information on structure, functions, catalyzed reactions and subcellular localization	Tissue localization	Reference(s)
SelR = SelX	●Cytosolic and nuclear protein with a molecular weight of 12 kDa. One Zn is additionally bound via 4 Cys residues ●Functions as methionine-sulfoxide-reductase: R-S (=O)-CH_3 + thioredoxin-$(SH)_2$→ R-S-CH_3 + H_2O + thioredoxin-S_2	liver, spleen, kidney, brain	161, 169 - 174
SelS	●First selenoprotein discovered using an in silico approach ●SelS has numerous glycosylation and phosphorylation sites ●SelS is also referred to as TANIS and its expression seems to correlate inversely with plasma glucose concen-tration ●Recent studies investigate a role of SelS in reverse transport of missfolded proteins into cytosol for ubiquitin-dependent degradation	numerous tissues cytosolic and membrane bound forms detected	161, 175 - 178
SelT	●A CysXXSec motif indicates redox properties	no information available yet	161, 168
SelV	●Identified using an in silico approach ●Shows partial homology to SelW ●CGLU motif suggests a redox-related function	liver, spleen, kidney, brain	161, 168
Selenoprotein W	●The ***W*** is derived from the fact that SelW is one of the missing seleno-proteins in Se deficient lambs with "***W***hite Muscle Disease" ●Antioxidant function not yet proved	mainly muscle tissue expression in nearly all mammalian tissues detectable cytosolic and membrane-bound forms detected	179 - 189
SelX = SelR	c.f. SelR		161, 169 - 174
SelY	●Seems to possess a function related to DIO2	liver, kidney	161
SelZ	●In literature SelZ is partially referred to as a splice variant of mitochondrial thioredoxin reductase (TrxR2)	expression in nearly all mammalian tissues	161, 168

2.3 Specific physiological functions of Se, Se compounds and functional selenoproteins

Observations on antidiabetic properties of ultra trace elements and trace elements were originally made for vanadium. The incubation of hepatocytes with vanadyl sulphate led to an increased glycogen synthesis in these cells [190]. For other cell types (adipocytes, skeletal muscle cells and fibroblasts) positive effects of vanadium compounds on glucose metabolism such as the stimulation of glucose uptake and oxidation and the induction of GLUT1 mRNA could be confirmed [191, 192, 193, 194, 195]. These effects could be attributed to an enhanced phosphorylation of signalling proteins downstream the insulin receptor like protein kinase c (PKC), phosphatidyl inositol-3-kinae (PI3K) and mitogen activated protein kinase (MAPK) [196]. Insulinomimetic properties of vanadate could also be found *in vivo* in type 1- and type 2-diabetic rats and mice when high doses (up to 5 mg per animal and day) of different vanadium compounds (vanadyl, vanadate, bis-[maltolato]-oxovanadium) were administered to the animals for several weeks [197, 198, 199, 200]. In more recent investigations it could be demonstrated that the insulinomimetic action of vanadium as the result of an enhanced phosphorylation of certain major proteins of the insulin signalling pathway is caused indirectly by an inhibition of PTPs (including PTP1B) rather than by a direct influence on phosphorylation. Vanadium compounds seem to bind to the catalytic active centre of PTP1B and therefore inhibit the enzyme activity [200, 201].

2.3.1 Effects of high selenate concentrations on glucose transport and uptake

Regarding insulinomimetic effects of Se, in the literature similar findings as for vanadate, are reported for selenate (Se oxidation state +VI). The first investigation into an insulin-like effect of selenate was made in an experiment with rat adipocytes [202]. Incubation of these cells with 100 µmol/L selenate resulted in a stimulation of glucose transport which was equipotent to that of 1nmol/L insulin. In contrast the incubation of adipocytes with selenite (Se oxidation state +IV) showed a distinctly lower stimulation of glucose transport. In this study the increase in glucose transport activity by selenate was attributed to the translocation of the glucose transporters (GLUT-1 and GLUT-2) to the membrane surface. This insulin-like effect of selenate on glucose uptake could also be confirmed in rat soleus muscle. In this experiment the incubation of the muscle with both sodium selenite and sodium selenate in increasing concentrations resulted in a markedly stimulated glucose uptake. A maximum stimulation was reached with a concentration of 100 mmol/L [203].

Antidiabetic effects of selenate could also be observed for the *in vivo* application to type 1 diabetic animals. When selenate was administered to rats and mice with streptozotocin induced type 1 diabetes orally or by intraperitoneal injection for 3 to 8 weeks in daily doses

up to half the lethal dose (≈ 3.5 mg/kg body weight) it lowered the elevated blood glucose to a level of non diabetic control animals [204, 205].

The oral treatment of mice with alloxan induced type 1 diabetes with a high dose of selenite (4 mg/kg body weight and day) however failed to reduce hyperglycemia in these animals. This observation suggests that fundamental differences in the intermediary metabolism of selenite and selenate lead to a differentiated influence of both Se compounds on pathways of nutrient metabolism [206].

2.3.2 Insulinomimetic effects of high selenate concentrations on gene expression related to glucose and fatty acid metabolism

In addition to glucose uptake into insulin sensitive tissues followed by glycolysis and glycogen synthesis, insulin fulfils a broad spectrum of other metabolic roles including facilitating the entry of amino acids into cells for the production of cellular protein. Moreover insulin controls the expression of a number of genes. Some insulin responsible genes are key enzymes associated with both carbohydrate and fatty acid metabolism, e.g. glycogen synthase, glucokinase, phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-diphosphatase (F-1,6-Dptase), fatty acid synthase (FAS) and glucose-6-phosphate-dehydrogenase (G6PDH) [207, 208]. Several studies have shown that both vanadate and selenate also possess insulinomimetic properties regarding to glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway. Vanadate was found to inhibit the expression of transfected chimeras of PEPCK in both FTO-2B and H4IIE rat hepatoma cells [209]. Similarly the oral administration of high selenate doses to type 1 diabetic rats partly normalized the changed expression of glyolytic and gluconeogenic marker enzymes (in diabetes the expression of glycolytic enzymes is down-regulated and gluconeogenic enzymes are up-regulated) to the level of non-diabetic animals. An up-regulation of glycolytic enzymes, in particular L-type pyruvate kinase, and for gluconeogenesis a down-regulation of PEPCK could be observed [210]. Regulation of the expression of lipogenic enzymes by selenate was also found as being similar to that of insulin. FAS and G6PDH activity were normalized in the livers of type 1 diabetic rats and hepatocytes. Selenate treatment of the diabetic animals or cultured rat hepatocytes restored the expression of both FAS and G6PDH, demonstrating that selenate was capable of stimulating lipogenesis in the liver [211, 212].

2.3.3 Further physiological effects of high selenate doses

A changed lipid metabolism in diabetic humans and animals with syndrom X is assumed to be one factor contributing to a higher risk of heart disease and apoplectic stroke. Against this background a study evaluated cardiac performance in streptozotocin induced type 1 diabetic

rats. The treatment of one rat group with supranutritional selenate doses improved glucose tolerance in these animals and normalized postprandial plasma glucose levels. Beside a high blood glucose concentration untreated diabetic rats developed increased left ventricular pressure. Treatment with selenate normalized the heart function. Moreover plasma lipid levels, triglycerides, cholesterol and free fatty acids were improved in selenate treated rats [205]. Thus a powerful influence of selenate on lipid metabolism seems to represent a further medical effect of high selenate doses [205, 213].

2.3.4 Cellular mechanism behind the antidiabetic = ”insulinomimetic” action of high selenate doses

All effects of insulin at the cellular level as described above are initiated by insulin binding to its plasma membrane receptor. Following insulin binding to the α subunit of the insulin receptor the protein changes its conformation and undergoes a multi-site phosphorylation in the cytosolic β subunit. By a subsequent phosphorylation of a number of endogenous substrates the insulin signal is spread and amplified by transmission to other signalling proteins. The insulin receptor substrate (IRS) family which includes IRS1, IRS2 and IRS3 (p60) is responsible for a number of insulin effects [214]. In contrast to insulin the insulinomimetics do not bind to the insulin receptor. Nevertheless the results of some studies show an increased phosphorylation of the β subunit of the insulin receptor and of its substrate IRS1. An increased tyrosine phosphorylation of the insulin receptor’s β subunit has been observed when cell cultures were incubated with vanadate. The insulinomimetic effect of selenate also seems to derive from an enhanced phosphorylation of certain compounds of the insulin signalling pathway. In the above mentioned early study with rat adipocytes not only could a stimulation of glucose transport be attributed to the incubation of these cells with selenate, but also important insight into the mechanism by which selenate develops its antidiabetic properties was given. After incubation of adipocytes the analysis of the whole cell lysate showed an enhanced phosphorylation of several cellular proteins with molecular weights of 170-, 95-, and 60 kDa. Thereby the 170 kDa protein presumably represented IRS1 and the 95 kDa protein was related to the β subunit of the insulin receptor [202]. In a study with NIH3T3 HIR 3.5 cells the effect of selenate on IRS1 phosphorylation could be confirmed [215]. In further experiments with 3T3L1 adipocytes and hepatocytes it could be demonstrated that beside IRS1 and the β subunit of the insulin receptor the p42 and p44-subunit of MAPK were also affected by an increased phosphorylation due to incubation with selenate in concentrations up to 1 mmol/L. Studies on general effects of insulin signalling proteins confirmed the the crucial role of PI 3-kinase for stimulation of DNA synthesis, glucose transporter translocation, regulation of glycogen synthase, glycogen synthase

kinase-3, the expression of PEPCK and G6PDH expression as well as GLUT-4-mediated glucose transport and membrane ruffling. One protein that has been identified as lying downstream of PI3-kinase is p70 S6 kinase [216]. Both S6 kinase and ribosomal S6 protein play an important role in the initiation of protein synthesis. In a study with primary adipocytes the incubation of these cells with selenate (100 µmol/L – 10 mmol/L) resulted in an increased phosphorylation of S6 kinase. In this study and in the above mentioned early study [202] the insulinomimetic effects of selenate (glucose uptake, increase in the phosphorylation of the β subunit of the insulin receptor and of S6 kinase) needed a certain incubation time until the onset of the reaction and an even longer period until a maximum response was achieved [217, 218].

On the whole the results of the studies mentioned so far have shown that selenate in high doses (*in vivo*: application of doses up to half the lethal dose = up to 3.5 mg Se as selenate/kg body weight, *in vitro:* incubation of living cells with 100 µmol/L – 10 mmol/L) has a distinct insulinomimetic effect which could be attributed to an increase in the phosphorylation of some major proteins of the insulin signalling pathway (c.f. Figure 4 in the "Discussion section").

2.3.5 Opposite effects of Se, Se compounds and functional selenoproteins on metabolic disorders

Opposite effects of Se regarding the development of insulin resistance and obesity have been shown in a recent study with transgenic mice overexpressing the selenoprotein GPx1. The decreased phosphorylation of the β subunit of the insulin receptor and of AKT in liver and muscle were attributed to GPx1 overexpression and led to the early onset of insulin resistance and obesity [219, 220].

Data of a human study support the hypothesis that there may be a coherence between GPx1 activity and diabetes incidence. Remarkable ethnic differences exist in erythrocyte GPx1 activity and populations with a higher GPx1 activity concurrently showed a higher incidence of gestational diabetes [221].

Another trial highlighting the inverse relation between H_2O_2-detoxifying enzymes and insulin resistance focussed on the effect of catalase overexpression. Catalase overexpressed mice with dietary induced insulin resistance had a slightly better protection against cardiac contractile dysfunction compared to wild type (WT) mice. However intrinsic insulin sensitivity in catalase overexpressed mice was significantly lower in comparison to WT mice, and it was based on a significantly increased expression of PTP1B due to catalase overexpression [222].

2.4 Protein tyrosin phosphatase 1B - its function and regulation and its role regarding insulin resistant diabetes and obesity

The PTPase family comprises a number of classes of functionally and structurally unrelated enzymes; it represents an important component of the protein-tyrosine phosphorylation/dephosphorylation mechanism, which regulates the level of tyrosine phosphorylation of a number of intracellular proteins. The so called cysteine based phosphatases (CBPs), which include protein tyrosine phosphatases (PTPs), dual-specificity phosphatases, low-molecular-weight PTPs, and the lipid phosphatase PTEN, all contain a nucleophilic catalytic cysteine within a conserved motif that enables these enzymes to dephosphorylate phosphoproteins or phospholipids [223, 224, 225].

Apart from the low molecular weight PTPs in recent years many studies have focussed on protein tyrosine phosphatase 1B (PTP1B) acting as a negative regulator of insulin signalling by its ability to dephosphorylate the insulin receptor substrates 1 and 2 as well as the intracellular β subunit of the insulin receptor [226].

Since obesity, insulin resistant type II diabetes and the metabolic syndrome represent disorders increasing in particular in industrial countries, research into molecular targets such as PTP1B linked to these diseases is gaining in importance [227, 228, 229].

In severe obese humans (body mass index > 40) a clear relation could be shown between obesity, insulin resistance and the activity of PTP1B in adipose tissue. Therapy of insulin sensitivity accompanied by weight loss led to a 20% reduction of PTP1B activity in adipose tissue [230]. Mice with a knockout of the PTP1B gene (PTP1B null) develop normally, with a lifespan comparable to their wild type littermates (WT) but they require only half the level of insulin for an optimum insulin signalling compared to WT mice [231]. When subjected to a high-fat diet PTP1B null mice were resistant to weight gain and remained insulin sensitive, whereas WT mice rapidly gained weight and became insulin resistant [232, 233]. Similar observations regarding insulin resistance were made for mice treated with an antisense oligonucleotide for PTP1B [233, 234, 235]. A strong inhibition of PTP1B enzyme activity along with an amelioration of diabetic symptoms could also be shown for vanadium compounds which act as potent PTP1B inhibitors [200, 201, 236]. Apart from the regulation of PTP1B activity by exogenously applied agents a number of recent investigations have focussed on the physiological inhibition of PTP1B in mammals via oxidation of the active site cysteine residue, Cys215, by H_2O_2 and reactive oxygen species. The activity could be recovered by the addition of dithiothreitol (DTT) which reduces the sulphenic acid intermediate and glutathionylated enzyme [237, 238]. Two investigations using mass spectrometry could further elucidate the stepwise oxidation of cysteine 215 in PTP1B by H_2O_2 [239, 240]. The cysteine sulphenic acid (PTP1B-SOH) may indeed undergo such reactions, although it can be oxidized further to other non-reducible derivatives like cysteine

sulphinic acid (PTP1B-SO_2H) and cysteine sulphonic acid (PTP1B-SO_3H). A possible way to prevent this oxidation of cysteine sulphenic acid to irreversibly oxidized forms is the reaction to the sulphenyl amide followed by the reaction with glutathione. The formation of a mixed disulfide between Cys-215 of PTP1B and GSH (or GSSG) is termed “glutathionylation”.

Also the direct reaction of the reduced Cys 215-SH with high concentrations of GSSG (> 25 mM) may induce the formation of glutathionylated PTP1B [241].
In mammalian metabolism one major prooxidative compound is H_2O_2 which is not only generated as a coproduct of the respiratory chain and some oxidoreductases but also after insulin binding to the insulin receptor in insulin sensitive tissues, presumably for the differential regulation of PTP activity [242, 243]. Thus the influence of Se and GPx1 on H_2O_2- and glutathione-concentration, representing just the critical metabolites in PTP1B regulation, strongly suggests a link between Se metabolism and pathways of intermediary metabolism.

2.5 Concluding remarks and relevance to the projected investigations

In industrial countries the number of patients suffering from obesity, insulin resistant diabetes and the metabolic syndrome is increasing permanently [227, 228, 229, 244]. Concomitantly in these countries the fortification of foodstuffs derived from plants and animals with vitamins, minerals and trace elements including Se by fertilization and animal nutrition is intensively practised [245 –252]. Health-conscious individuals frequently consume multivitamin and/or Se supplements in order to optimize their antioxidant defense and to obtain a better protection against several cancer types [253].

Information from literature available to date suggests that Se in addition to its antioxidative function plays a pivotal role in the regulation of metabolic processes.

- On the one hand high supranutritional selenate doses effect antidiabetic properties.
- On the other hand an exaggerated detoxification of H_2O_2 by glutathione peroxidase 1, representing the best characterised selenoprotein, seems to accelerate the development of insulin resistant diabetes and obesity. This even negative aspect of Se supply may be related to its influence on the regulation of H_2O_2 and glutathione representing the critical metabolites modulating the activity of insulin antagonistic protein tyrosine phosphatases.

The aim of the present studies consequently was to examine molecular coherences and mechanisms behind both physiological properties of Se in different rodent species.

3. Insulin-sensitizing and antidiabetic features of high supranutritional selenate doses

3.1 Introduction to chapter 3

At the beginning of the experiments in this study little information on antidiabetic effects of Se, Se compounds and selenoproteins existed. The studies available had been carried out only with type I diabetic animals and with tissue cultures. Moreover insulinomimetic features of Se have been described exclusively for selenate (Se oxidation state: +VI) [202, 203-206, 210, 212].

- No information was available on possible insulinomimetic or insulin sensitizing properties of selenate in animals with insulin resistant type II diabetes and obesity.
- Information on the molecular mechanisms behind the insulinomimetic benefits of selenate in relation to mammalian Se metabolism was lacking.
- Moreover no investigations on a distinct differentiation of the insulinomimetic properties of selenate in comparison to other Se compounds on obesity, insulin resistance and diabetes could be found.

- The aim of the first part of the present studies consequently was to examine if selenate also evolves insulinomimetic features in type II-diabetic animals. For this purpose two experiments were carried out with C57BL/KsOlaHsd-Lepr db mice (dbdb mice) with a defective leptin receptor, featuring severe symptoms of obesity and insulin resistant type II diabetes.
- The second main topic of the studies with dbdb mice was to examine whether insulinomimetic properties are only derived from selenate (Se oxidation state: +VI) or if other inorganic Se compounds like selenite (Se oxidation state: +IV), frequently used as Se supplements for diets of laboratory animals and livestock, also have insulinomimetic effects.
- The third aim of the dbdb mouse studies was to investigate molecular mechanisms behind insulinomimetic effects of selenate in type II diabetic animals.

The experimental setup of the two dbdb mouse trials as well as the results of these experiments and a discussion of the results is given in the following three publications:

1. Müller A.S., Pallauf J. and Rafael J. (2003): The chemical form of selenium affects insulinomimetic properties of the trace element: Investigations in type II diabetic dbdb mice. J. Nutr. Biochem. 14, 637 – 647

- [publication No. 254 in the Literature Index]

2. Müller A.S., Erika Most and Pallauf J. (2005): Effects of a supranutritional dose of selenate compared to selenite and selenium deficiency on insulin sensitivity in type II diabetic dbdb mice. J. Anim. Physiol. Anim. Nutr. 89, 94 – 104

- [publication No. 255 in the Literature Index]

3. Müller A.S. and Pallauf J. (2006): Compendium of the antidiabetic effects of supranutritional selenate doses. *In vivo* and *in vitro* investigations with type II diabetic dbdb mice. J. Nutr. Biochem. 17 (8), 548 - 560

- [publication No. 256 in the Literature Index]

The above mentioned publications can be found as the “Attachment 2” of this work, following page 85.

4. Potentially critical functions of Se regarding obesity, insulin resistance glucose- and lipometabolism

4.1 Introduction to chapter 4

During the experiments with dbdb mice it became evident that antidiabetic effects of Se are restricted to the oral application of high supranutritional selenate doses. The fact that in industrial countries on the one hand the incidence of diabetes is permanently increasing, and on the other hand a fortification of foodstuffs with trace elements like Se is also widely practised, raised the question if a permanent surplus of dispensable Se may be critical for the development of diabetes. To investigate this hypothesis two studies with healthy growing rats were carried out. Shortly after the completion of the data analysis of the first rat study, the first critical findings on the effects of Se on insulin resistant diabetes and obesity were published [219, 220, 221].

The few results published regarding this undesirable aspect of Se were however obtained either from a non-physiological animal model with an overexpression of the selenoprotein GPx1 [219, 220] or they represented a merely statistical data analysis [221]. Moreover these investigations did not provide sufficient explanations of mechanisms by which Se may develop undesirable influences on insulin resistant diabetes and obesity.

A physiological model examining the effects of a permanent moderate surplus of Se on molecular pathways linked to insulin resistant diabetes and obesity was lacking.

- Thus the aim of the second part of this work consequently was to critically examine cellular pathways by which Se can potentially influence the development of obesity, insulin resistance and type II diabetes even negatively.

Therefore two trials were performed with healthy growing rats fed Se at the recommended dietary level and in slightly supranutritional doses (5-and 10-fold the recommended daily amount).

- To find out critical cellular triggers regarding insulin resistant diabetes and obesity influenced by Se, a gene expression profile was carried out using microarray analysis.
- The second main focus of the analyses was the examination of the regulation of PTP1B by Se. In current literature this insulin antagonistic phosphatase is discussed as one promising target for both the development and therapy of insulin resistance and obesity. Since Se and GPx1 are involved in the regulation of glutathione and H_2O_2 in tissues, representing those central metabolites in the modulation of PTP1B, the idea suggested itself to examine if PTP1B may be one molecular target triggering undesirable influences of Se on insulin resistant diabetes and obesity.

The experimental design of both experiments, the results of these experiments and a discussion of the results is given in the following two publications:

1. Müller A.S., Astrid Bosse and Pallauf, J. (2006): Selenium an ambivalent factor in diabetes? Established facts, Recent findings and Perspectives. Current Nutrition and Food Science 2 (2), 151 – 168 (Invited Review: Combined review and original contribution containing a depiction of new experimental results)

- [publication No. 257 in the Literature Index]

2. Mueller AS, Bosse AC, Most E, Klomann SD, Schneider S and Pallauf J. (2007): Selenium a risk factor for diabetes development ? The regulation of PTP1B may be one part of the puzzle (submitted to J Nutr Biochem)

- [publication No. 258 in the Literature Index]

The above mentioned publications can be found as the “Attachment 2” of this work, following page 85.

Additional information regarding publication 2: The latter publication is meanwhile in press in the Journal of Nutritional Biochemistry under a changed title. During the review process the reviewers desired changing of the title. The publication now reads:
Mueller AS, Bosse AC, Most E, Klomann SD, Schneider S, Pallauf J.
Regulation of the insulin antagonistic protein tyrosine phosphatase 1B by dietary Se studied in growing rats. J Nutr Biochem. 2008 Jul 3 [Epub ahead of print]
http://www.ncbi.nlm.nih.gov/pubmed/18602818?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

5. General discussion and future perspectives

5.1 Antidiabetic effects of high supranutritional selenate doses

5.1.1 Knowledge concerning antidiabetic effects of high supranutritional selenate doses at the start of the present studies

When starting the present studies on effects of Se on type II diabetes with dbdb mice only little information regarding antidiabetic effects of high supranutritional selenate doses (Se oxidation state +VI) were available. Furthermore all these investigations were carried out in tissue cultures and with animals with alloxan –or-streptozotocin-induced type I diabetes [202–205, 210-213, 217, 218].

No information on possible antidiabetic effects of high supranutritional selenate doses was available for animal species with type II diabetes and insulin resistance.

Moreover no results on a distinct differentiation of the insulinomimetic properties of selenate (oxidation state +VI) in comparison to other inorganic Se compounds (e.g. selenite: oxidation state +IV) were reported before the start of the present studies.

5.1.2 Antidiabetic effects of high supranutritional selenate doses in a rodent model with severe insulin resistant diabetes: New investigations into the molecular mechanism behind these effects

In the first study with adult male dbdb mice a group with Se deficient nutrition for 10 weeks was compared to two experimental groups receiving the same diet in combination with 15% of their individual half lethal dose of selenite (Se +IV) or selenate (Se +VI). These Se supplements amounted to about 15 to 20-fold the recommended dietary level. At the beginning of the experiment the animals already had an established severe type II diabetes, indicated by a very high starved blood glucose concentration (about 25 mmol/L = 450 mg/dL). At the end of the trial characteristic changes in the activity of some glycolytic and gluconeogenic enzymes towards a normalization of metabolism (increase in glycolysis and decrease in gluconeogenesis) could be analyzed exclusively in selenate treated animals. These results are in accordance with prior examinations with type I diabetic animals. The changes regarding glycolysis and gluconeogenesis measured in the first dbdb mouse trial were not as distinctive as reported in prior examinations [204, 212]. This observation may result from the lower Se doses used in this trial compared to other investigations (15% of the LD_{50} vs. 50 to 100% of the LD_{50}). Precisely the use of these lower doses was intended to test if antidiabetic effects could already be obtained with lower and thus more physiological selenate concentrations.

- The most important and new finding of the first dbdb mouse study was the observation that the application of high supranutritional selenate doses led to an amelioration of insulin sensitivity in the type II diabetic mice, as found by whole body

insulin sensitivity tests [249]. Selenite treatment even caused an impaired diabetic status and deteriorated insulin sensitivity. The ineffectiveness of selenite in diabetes therapy was also found in a study with alloxan treated type I diabetic mice, which was published soon after the results of the first dbdb mouse trial [206].

The amelioration of insulin sensitivity by therapy with high supranutritional selenate doses was the basis for the second study with female growing dbdb mice focussing on the examination of molecular mechanisms behind the insulin sensitizing effects of selenate.

In prior studies with tissue cultures and type I diabetic animals the insulinomimetic effects of selenate were linked to an increased phosphorylation of certain proteins of the insulin signalling pathway (c.f. for details 2.3.4) [202, 217, 218]. The proteins with increased phosphorylation investigated in the above mentioned studies are numbered with 1) and 2) and indicated by orange arrows in Figure 4.

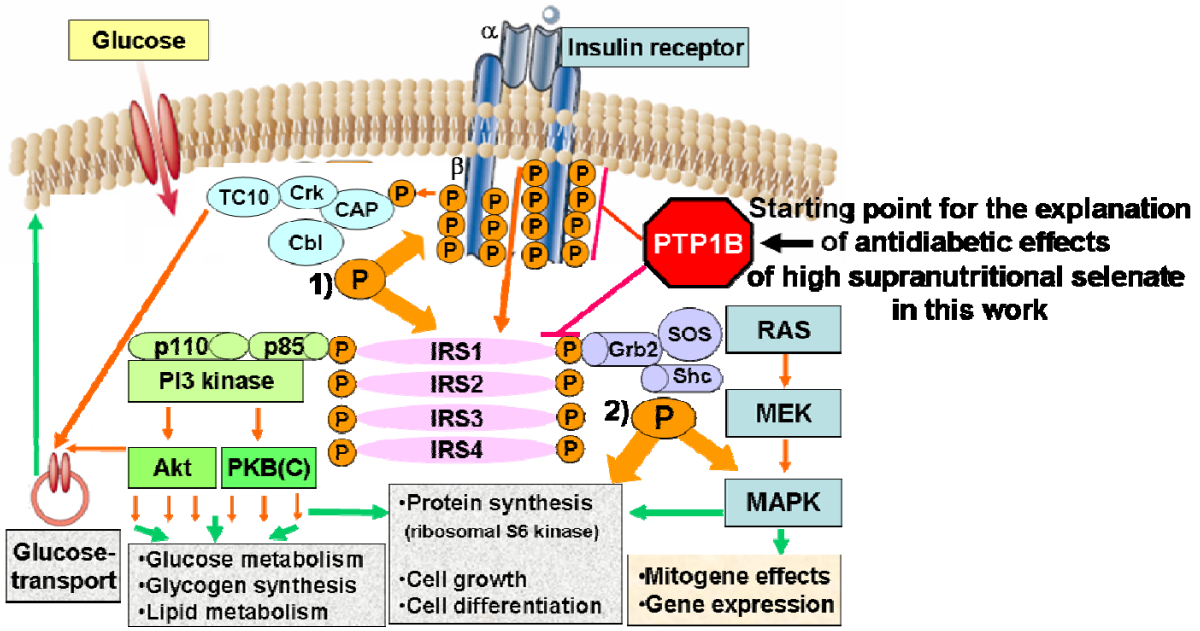


Figure 4: ***Main pathways of the insulin signalling cascade and interfaces with antidiabetic effects of high supranutritional selenate doses***

The experimental conditions in the second trial with growing female dbdb mice were similar to those in the first dbdb mouse trial with adult male animals [254, 255]. In this experiment growing female animals were chosen deliberately because diabetes development in female dbdb mice is not as rapid as in male animals [259]. Thus in contrast to the first trial in which adult male mice with fully established diabetes served as experimental animals in the second trial growing animals were chosen in order to test the retarding effect of selenate on diabetes

development. Furthermore in the second dbdb mouse trial slightly higher selenite- and selenate concentrations (up to 35% of the LD_{50} = about 30 to 35-fold the recommended dietary amount) were applied to guarantee sufficient antidiabetic effects. In comparison to prior studies with type I diabetic animals these doses were however still relatively low. The results of the first dbdb mouse trial could be corroborated by the data of the second trial. Insulin sensitivity could be maintained till the end of the experiment in selenate treated mice whereas a distinct impairment of insulin sensitivity was measured in Se deficient and selenite treated mice.

- Besides very powerful influences of selenate on the reduction of gluconeogenesis and on lipid metabolism, a central result of the second dbdb mouse study was the finding that high supranutritional selenate doses reduce the activity of insulin antagonistic protein tyrosine phosphatases (PTPs) [255, 256].

This result is a novel finding, which has not been reported previously, and it is concurrently not inconsistent with prior results. The reduction of PTP activity provides a plausible explanation for the maintenance of insulin sensitivity due to the treatment of insulin resistant animals with high supranutritional selenate doses (Figure 5). Since PTPs and in particular PTP1B act as potent inhibitors of insulin signalling, the inhibition of these enzymes increases insulin sensitivity and simultaneously effects an increase in the phosphorylation of downstream proteins of the insulin signalling pathway. Effects on metabolic pathways like glycolysis, gluconeogenesis and lipid metabolism finally arise as the consequence of PTP inhibition and the subsequent increased phosphorylation.

Thus in accordance with prior investigations metabolic effects of high supranutritional selenate doses [203–205, 210-212] are mediated by an increase in the phosphorylation of central proteins in the insulin signalling pathway [202, 217, 218], but in consideration of the present results they represent the consequence of a strong inhibition of PTPs rather than reflecting a direct influence of selenate on protein phosphorylation (Figure 4).

- The second central finding regarding insulin sensitizing and antidiabetic effects of high supranutritional selenate doses derives from the results of the “PTP *in vitro* inhibition test” (Figure 5) [255, 256].

The results of this test were initially surprising, since selenate (Se +VI) per se did not inhibit PTP activity *in vitro*. A strong inhibition of PTP activity could be found instead for the selenite

oxidation state +IV [derived from: a) selenate, reduced with HCl, b) selenite, c) selenious acid]. A lower, but still distinct inhibition of PTPs could be obtained with selenotrisulfides. The coherence between the Se compound used and PTP inhibition could be clearly displayed by correlation and regression analyses (Table 2, Figure 5).

With the exception of selenate a distinctly negative correlation could be found between the Se concentration in the assay and PTP inhibition for Se +IV compounds and for selenotrisulfides (oxidation state – I). The slopes in square regression analyses were however distinctly steeper for the Se +IV compounds compared to selenotrisulfides, confirming their lower inhibition.

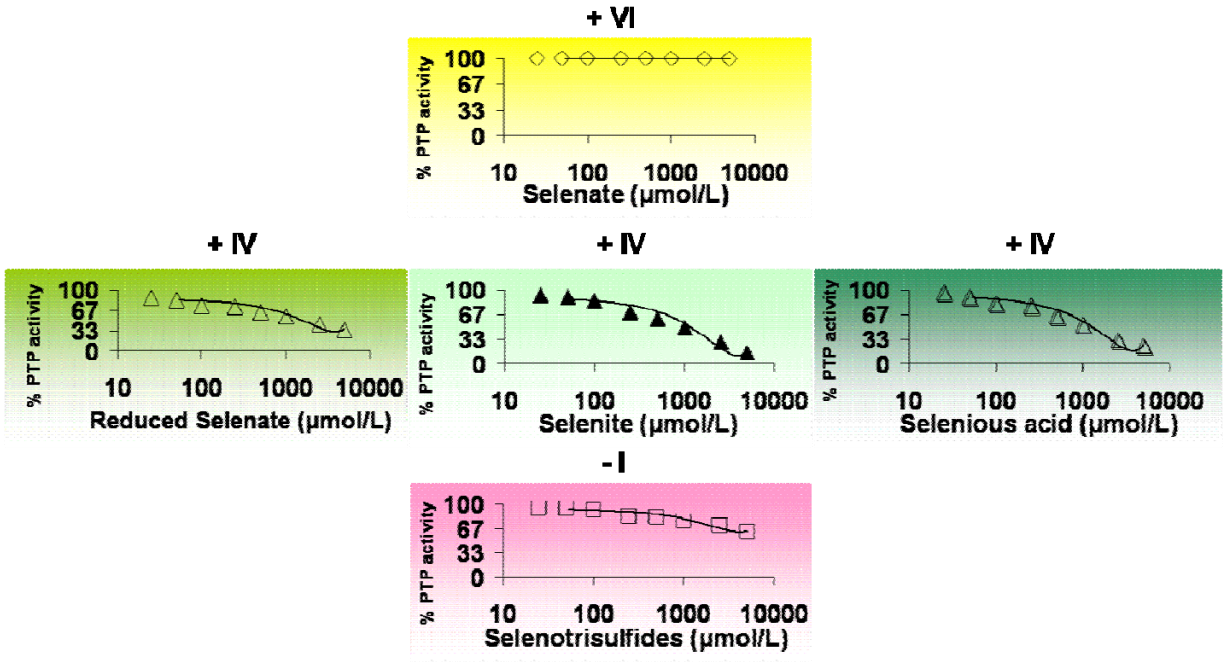


Figure 5: ***In vitro inhibition kinetics of different Se compounds on PTP activity in dbdb mouse liver cytosol***

A plausible explanation for the results of the *in vitro* inhibition assay is founded on fundamental differences in mammalian Se metabolism. Se from selenite (+IV) and selenate (+VI) is absorbed by different mechanisms [13]. Selenite either reacts with thiols like glutathione prior to its absorption and enters the peripheral organs in the form of selenotrisulfides (oxidation state: - I) or it is reduced in the erythrocytes to the selenide oxidation state –II and delivered to peripheral organs bound to albumin [14, 15, 16]. In contrast unmodified selenate can be detected in the bloodstream and in peripheral tissues [17, 19]. During successive selenate reduction both thiol reactive oxidation states as selenite (+IV) and selenotrisulfides (–I) can be formed intermediately *in vivo*.

Table 2: Correlation- and regression-analyses for the in vitro inhibition kinetics of different Se compounds on PTP activity in dbdb mouse liver cytosol

Relation examined	**Se compound and concentration : PTP inhibition**
Selenate (Se +VI)	
Pearson correlation	0
	p<0.001
Linear regression	y = 100
	R^2 = 1.0
Selenate reduced with HCl (Se + IV)	
Pearson correlation	-0.854
	p<0.003
Square regression	$4 \times 10^{-6} x^2$ - 0.0278 x + 84.688
Selenite (Se + IV)	
Pearson correlation	-0.895
	p<0.001
Square regression	$5 \times 10^{-6} x^2$ - 0.0401 x + 89.414
Selenious acid (Se + IV)	
Pearson correlation	-0.887
	p<0.001
Square regression	$5 \times 10^{-6} x^2$ - 0.0392 x + 91.310
Selenotrisulfides (Se – I)	
Pearson correlation	-0.888
	p<0.001
Square regression	$2 \times 10^{-6} x^2$ - 0.0153 x + 93.291

In vivo selenate application therefore matches *in vitro* effects of the selenite- and selenotrisulfide oxidation states +IV and –I. But an effective PTP inhibition by oral selenate application presumably also depends on the kind of application: As practised in the dbdb mouse trials a quick and intermittent flooding of selenate to the peripheral organs by tube feeding can be assumed as more effective than feeding the Se as a dietary component, because the organism has then a rapid need for selenate reduction.

As biochemical mechanisms behind PTP inhibition through oral selenate application mimicked by *in vitro* incubation with selenite the following two pathways are likely:

1. Se IV compounds (derived from selenate feeding or *in vitro* incubation with selenite) react with the active site SH group of PTP1B and form a cysteine-selenodisulfide-bond, leading to PTP1B inhibition (Figure 6A).
2. Se IV reduction leads to the formation of superoxide radicals [260, 261] which attack the active site cysteine of PTP1B and effect its oxidation to the inactive sulfenic acid [262, 263] as shown in Figure 6B.

A)

B)

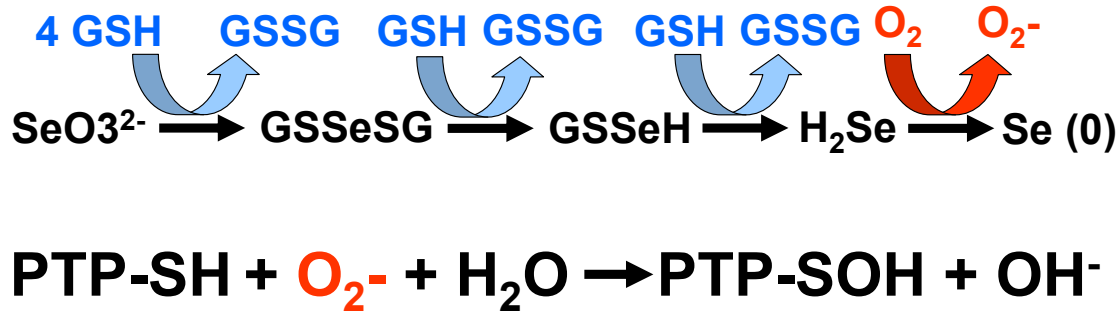


Figure 6: ***Presumed pathways of PTP1B inhibition by oral selenate application or in vitro incubation with selenite***

A) At the expense of glutathione selenite directly reacts with the active site SH-group of PTP1B

B) At the expense of glutathione selenite generates superoxide radicals, which oxidize the active site SH-group of PTP1B to a sulphenic acid

Thus it is plausible that an effective PTP inhibition can only be achieved in the presence of the thiol reactive selenite oxidation state +IV. A distinctly lower PTP inhibition seems to take place through the selenotrisulfide oxidation state –I. The inhibitory effect of both Se compounds on PTP activity is based on their need for thiols for their further reduction.
Future investigations using mass spectrometry and radical trappers could be helpful for the further explanation of the exact reaction mechanisms by which high doses of oral selenate can inhibit PTP1B activity.

In conclusion both dbdb mouse studies [254, 255, 256] could contribute to the clarification of molecular mechanisms by which the oral application of high supranutritional selenate to type

II diabetic animals with insulin resistance can perform antidiabetic and insulin sensitizing effects (Figure 7). An increased insulin sensitivity obtained by oral selenate supply thereby involves selenate reduction during Se metabolism and the subsequent inhibition of PTPs by intermediary formed Se IV compounds. Thus the increased phosphorylation of other proteins of the insulin signalling pathway is more likely a consequence of PTP inhibition than a direct influence of Se on protein phosphorylation.

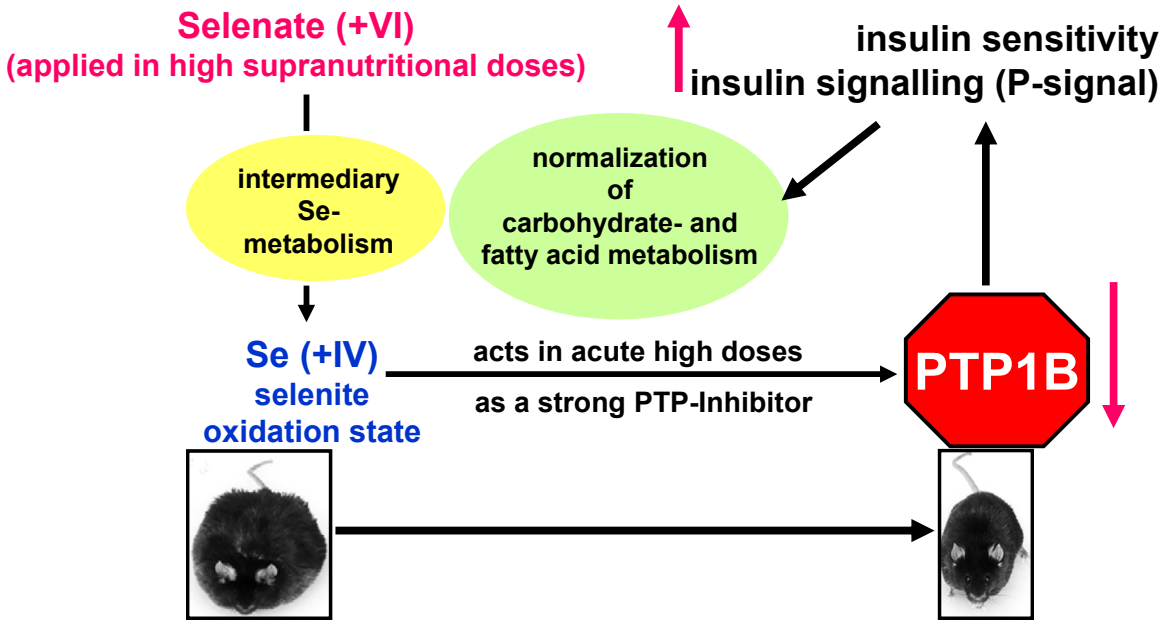


Figure 7: Outline of the insulin sensitizing and antidiabetic effects of high supranutritional selenate doses

5.1.3 Future perspectives for the use of high supranutritional selenate doses as a therapeutic agent in the treatment of insulin resistance and type II diabetes

In conclusion the present trials with type II diabetic dbdb mice could elaborate the following novel findings on antidiabetic effects of Se:

1) Selenium develops antidiabetic effects also in type II diabetic species and they are based on an amelioration of insulin sensitivity.
2) Diabetes therapy with selenium is restricted to the oral application of high supranutritional selenate doses, and the antidiabetic effects of selenate are related in particular to mammalian Se metabolism
3) Contrary to prior studies it could be demonstrated in a type II diabetic animal model that insulinomimetic and insulin sensitizing effects of selenate are based on an inhibition of PTPs rather than on a direct influence of phosphorylation of downstream proteins in the insulin signalling pathway

The safety of high supranutritional selenate doses, as required for antidiabetic effects, however has so far only been tested in tissue cultures and in animal models and it is still questionable if selenate application in therapeutical and thus physiological doses can be relevant for diabetes treatment in humans. The present studies with dbdb mice however could contribute to a considerably better understanding of the molecular mechanisms by which selenate attains antidiabetic effects.

5.2 Potentially critical functions of Se regarding insulin resistant diabetes and obesity

During the research on antidiabetic and insulin sensitizing features of Se selenate it appeared that these effects, based on protein tyrosine phosphatase inhibition, represent a merely biochemical effect of high supranutritional selenate doses (Se +VI). Due to an increasing health awareness in industrial countries the fortification of foodstuffs with vitamins, minerals and trace elements including Se by fertilisation, animal nutrition or pure addition is widely practised to improve product quality and to comply with consumer demands for functional food [245-253]. On the other hand an increased incidence of metabolic disorders like obesity, diabetes and syndrome X can be observed in these very countries [227, 228, 244], and it raises the question if a permanent oversupply with de facto health-supporting substances may have undesirable properties regarding the above mentioned disorders. The first reports regarding such critical effects of Se [219, 220, 221] were published shortly after the evaluation of the data from the first rat study - scanning molecular mechanisms behind undesirable effects of Se on insulin resistant diabetes - was completed. In the following section a brief outline of the essential points from studies dealing with critical effects of Se on insulin resistant diabetes and obesity is given:

5.2.1 Findings on critical functions of Se regarding insulin resistant diabetes and obesity in the most recent literature

A U.S. study, published in 2003 [221] examined the coherence between erythrocyte glutathione peroxidase activity and gestational diabetes in 408 women. In this study two major findings were pointed out:

1. Women from different ethnic groups which were included into this project showed significant differences in their erythrocyte GPx1 activity. Significantly higher erythrocyte GPx1 activities in African-American women compared to Caucasians at the beginning of pregnancy were maintained till the third trimester. During pregnancy erythrocyte GPx1 activity in all three ethnic groups increased. The examination of nutritional habits in the different ethnic groups revealed that African-American women had a statistically significantly higher dietary fat intake, and thus additionally a highly positive correlation between dietary fat intake and erythrocyte GPx1 activity could be evaluated.
2. Further data analysis in this study showed the existence of a significantly positive correlation between the height of erythrocyte GPx1 activity and the degree of insulin resistance during development of gestational diabetes. Women with a high GPx1 activity also featured higher basal insulin- and reactive-C-peptide-levels to compensate for their insulin resistance.

Another interesting study, published in 2004 [219, 220], found an accelerated development of obesity and insulin resistant diabetes for mice with an overexpression of glutathione peroxidase 1. One group of mice in this experiment had an overexpression of whole body GPx1 (OE), whereas the control group consisted of animals of the black 6 wild type (WT). The mice of both groups were fed a diet containing a sufficient amount of 0.4 mg Se/kg diet for 16 weeks (week 8 after birth till week 24). Thus an alimentary Se deficiency could be excluded as a factor responsible for changes in GPx1 activity.
OE mice had a 21% higher GPx1 activity in the liver and 3-fold higher GPx1 activity in skeletal muscle compared to WT mice. OE mice featured a 37% higher body weight compared to WT mice and they had a 20% higher absolute whole body fat content. By week 24 basal insulin concentrations were significantly higher in OE mice compared to their WT companions. At the end of the experiment insulin resistance in OE mice was significantly higher than in WT mice.
As the molecular cause for the increased insulin resistance the authors could show a decreased phosphorylation of the β subunit of the insulin receptor and of Akt a downstream protein involved in insulin signal transduction (c.f. Figure 4).

A further study with mice, published in 2006 [222], did not directly examine the influence of glutathione peroxidase on insulin resistance - but instead the influence of catalase, the second main enzyme involved in cellular H_2O_2-detoxification. Feeding wild type mice a diet with a high sucrose content inherently led to the advanced development of insulin resistance, accompanied by an increased expression of PTP1B compared to mice fed a diet based on starch. In this experiment two further groups of transgenic animals had an overexpression of catalase (sucrose fed + catalase overexpression, starch fed + catalase overexpression). In particular sucrose fed mice with catalase overexpression featured the highest PTP1B within the experimental groups. The antioxidative effect of the H_2O_2-detoxifying catalase could indeed partially reverse cardiac contractile dysfunction by lowering oxidation of contractile proteins, but on the other hand it increased PTP1B expression and with it the intrinsic insulin resistance.
Very recent results from two U.S. studies, published in 2007 [264, 265] with sizable human populations (NHANES III trial = Third National Health and Nutrition Examination Survey and NPC trial = Nutritional Prevention Of Cancer) independently found a distinct positive correlation between serum Se concentration and the incidence of type II diabetes. Data analysis in the NHANES III trial (8.876 participants) was based on conventional nutrition whereas the NPC trial was a randomized study in which one group was supplemented with 200 µg Se daily in order to test prevention against non melanoma skin cancer (600 participants). The control group (602 participants) received a placebo for 7.7 years.

The data of the NHANES III trial revealed that Se status in U.S. adults is distinctly higher in comparison to other populations. After assignment of the data to quintiles participants from quintile 5 (serum Se ≥ 137.66 mg/L) had a 1.57-fold higher diabetes incidence compared to participants from quintile 1 (serum Se ≤111.62 µg/L). The results from the NPC trial were even stronger compared to the NHANES III trial. The median value of serum Se concentration from those Se supplemented participants and from the placebo group was 113.4 µg/L. Diabetes incidence in the residual group with a serum Se concentration beyond the median (>113.4 µg/L) was 2.5-fold higher compared to the residual group with a serum Se concentrations below the median (≤ 113.4 µg/L). When the data were subdivided into tertiles, diabetes incidence was even 2.7-fold higher in the third tertile (serum Se >121.6 µg/L) compared to the first tertile (serum Se ≤105.2 µg/L).

The few results published regarding undesirable aspects of Se on insulin resistant diabetes and obesity however provided only very little information concerning two major aspects.

1) Data of all the above mentioned studies were obtained either from non-physiological animal models with the overexpression of a particular gene [219, 220, 222] or they represented a merely statistical data analysis [221, 264, 265].
2) Moreover these investigations did not provide satisfactory explanations of the molecular mechanisms by which Se may have an undesirable influence on insulin resistant diabetes and obesity.

Physiological models examining the effects of a permanent moderate surplus of Se on molecular pathways linked to insulin resistant diabetes and obesity were lacking.

5.2.2 New approaches in the present studies to explain mechanisms behind critical functions of Se regarding insulin resistant diabetes and obesity

In the present studies consequently two nutrition physiological approaches [259, 260] with healthy growing rats were used to investigate molecular mechanisms behind undesirable effects of Se on the development of insulin resistant diabetes and obesity:

1. A microarray screening was carried out to evaluate genes which may mediate undesirable effects of additional Se supply on the development of insulin resistant diabetes and obesity
2. A detailed study of PTP1B regulation by moderately supranutritional Se supply was carried out, since PTP1B was one of the critical genes, detected by microarray analysis. Moreover PTP1B represents one powerful enzyme contributing to insulin resistance and obesity, and PTP1B inhibition is discussed as one promising aim in the therapy of the disorders mentioned. Another reason for the examination of PTP1B regulation by Se was its physiological regulation by H_2O_2 and glutathione, representing these critical metabolites which are also influenced by glutathione peroxidase and intermediary Se metabolism. Due to its position at the top of the insulin signalling pathway PTP1B is finally an interesting target protein, since it can influence the regulation of a number of downstream genes of glucose and lipid metabolism.

For both rat trials the experimental design was comparable. Weaned albino rats of the institutes own strain HK51 (initial mean body weight in trial 1: 62.8±3.95 g; in trial 2: 61.0±2.96) were fed diets based on Torula yeast for eight weeks. In both trials one group received a Se deficient basal diet, and the diets of the further 6 groups were supplemented with both sodium selenite and sodium selenate to obtain final Se concentrations of 0.2, 1.0 and 2.0 mg Se/kg diet.

Trial 1 served as the basis to obtain information on the differential regulation of genes by both inorganic Se compounds (selenite or selenate) in comparison to a group with a short term Se deficiency. Therefore a microarray screening (MWG Rat 10k Array) with pooled liver RNA samples from 5 animals of the groups 0Se, 0.2 Selenite, 0.2 Selenate, 1.0 Selenate and 1.0 Selenate was carried out. With this microarray technology expression changes in nearly 10,000 rat genes can be examined simultaneously.

- One main result of the microarray screening revealed that the influence of selenate on differential gene expression was by far higher than that of selenite.

Table 3 outlines an overview of the expression data. The influence of selenite- and selenate-feeding at the two levels (0.2, and 1.0 mg Se/kg diet) compared to a short term Se deficiency (0Se) is displayed at different factors for the expression differences combined with different significance levels for gene regulation.

Table 3: ***Summary of the impact of selenite and selenate at two different dietary concentrations (0.2 and 1.0 mg Se) on differential gene expression in rat liver compared to short term Se deficiency***

Comparison	Power Factor: 1.5 p<0.05	Power Factor: 2.0 p<0.05	Power Factor: 2.0 p<0.01	Power Factor: 2.5 p<0.05	Power Factor: 2.5 p<0.01
0.2 Selenite: 0Se					
Total:	**117**	**72**	**24**	**45**	**18**
Up:	**73**	**51**	**15**	**34**	**13**
Down:	**44**	**21**	**9**	**11**	**5**
0.2 Selenate: 0Se					
Total:	**951**	**344**	**211**	**272**	**159**
Up:	**662**	**233**	**132**	**234**	**132**
Down:	**289**	**111**	**79**	**38**	**27**
1.0 Selenite: 0Se					
Total:	**261**	**81**	**29**	**41**	**15**
Up:	**190**	**57**	**18**	**29**	**9**
Down:	**71**	**24**	**11**	**12**	**6**
1.0 Selenate: 0Se					
Total:	**819**	**491**	**272**	**317**	**193**
Up:	**548**	**345**	**172**	**251**	**146**
Down:	**271**	**146**	**100**	**66**	**47**

- The second central finding of the microarray experiment was that a number of genes involved in the regulation of intermediary metabolism signalling processes of the insulin pathway, and genes of neuropeptides participating in the regulation of appetite and energy homeostasis showed a changed expression due to Se supplementation [259].

A selection of these genes was published in a review presenting also the latest findings from the first rat study [257]. Table 4 gives a more comprehensive outline of genes regulated by selenate supplementation which could be critical for the development of insulin resistant diabetes and obesity. The selected genes may represent starting points for future research investigating the critical role of moderate supranutritional Se supplementation, exceeding the needs for selenoprotein synthesis. The literature sources given in Table 4 substantiate the coherence between the genes regulated as well as the direction of regulation (up-or down-

regulation) and their critical role in the development of insulin resistant diabetes and/or obesity.

As can be seen in Table 4 the expression changes measured for the functional selenoproteins are in accordance with literature. The low rank of GPx1 and the high rank of GPx4 in the hierarchy of functional selenoproteins due to a lack in dietary Se supply were clearly reflected by the microarray data [37, 126, 128].

Contrary to the application of high supranutritional selenate doses which reduced the mRNA concentrations of gluconeogenic enzymes of type II diabetic animals to the level of non-diabetic controls [256, 257], selenate feeding at the recommended dietary level (0.2 mg Se/kg diet) and at slightly supranutritional doses (1.0 mg Se/kg diet) led to an up-regulation of the mRNA-levels of the gluconeogenic enzymes glucose-6-phosphatase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase compared to group 0Se with a short term Se deficiency. Thus long term Se supplementation above the needs may redound to endogenous glucose synthesis and an increase in blood glucose levels.

The distinct up-regulation of the mRNA´s for protein tyrosine phosphatase εC, protein tyrosine phsophatase 1B and for the regulatory subunit of protein tyrosine phosphatase 2A [275-279, 335-337], as an assembly of genes participating in the counter-regulation of insulin signalling and triglyceride synthesis could explain the development of insulin resistance and obesity due to long term Se supply above the needs. Coherences and molecular mechanisms by which an up-regulation of this mentioned gene assembly can contribute to the development of insulin resistance and obesity are explained in detail in Figure 11.

Table 4: ***Influence of selenate at two different dietary levels (0.2 and 1.0 mg Se/kg diet) on the expression of genes critical for the development of insulin resistant diabetes and obesity compared to short term Se deficiency***

(Table legend)

Factors for gene regulation ≥ 1.5 and an error probability < 0.05 are displayed in bold font Genes displayed in **black writing** are up-regulated ↑ Genes displayed in **red writing** are down-regulated ↓
Genes displayed on a white background belong to the functional selenoproteins as reference genes, or they represent genes regulated with a factor < 1.5 or an error probability > 0.05
Genes displayed on a yellow background represent genes associated with intermediary metabolism
Genes displayed ona green background are phosphatases or kinases involved in the regulation of metabolic processes
Genes displayed on a blue background represent neuropeptides and other factors involved in the regulation of appetite, satiation and other endocrine functions
Genes displayed on a pink background represent cytokines involved in the regulation of metabolic processes

Table 4: ***Influence of selenate at two different dietary levels (0.2 and 1.0 mg Se/kg diet) on the expression of genes critical for the development of insulin resistant diabetes and obesity compared to short term Se deficiency***

No.	Regulated gene	x-fold regulation 0.2 Selenate : 0Se	Error probability (t - Test)	x-fold regulation 1.0 Selenate : 0Se	Error probability (t - Test)	Literature
	Glutathione peroxidase 1	6.62	0.000	5.88	0.000	
	Phospholipid hydrop. glutathione peroxidase 4	1.32	0.014	1.31	0.016	
	Selenoprotein P	2.11	0.000	1.94	0.000	
1	Acyl-coenzyme a : cholesterol acyltransferase (SOAT1)	**3.44**	**0.018**	**3.00**	**0.041**	266, 267
2	Advanced glycosylation end product receptor (RAGE)	1.41	≤ 0.100	1.50	≤ 0.100	268, 269
3	Agouti related protein precursor (AGRP)	**6.17**	**0.017**	**3.00**	**0.002**	270
4	APO E	**4.50**	**0.004**	**4.76**	**0.002**	271
5	Apolipoprotein A 5	**1.63**	**0.002**	**1.81**	**0.001**	272
6	Branched-chain alpha-ketoacid dehydrogenase kinase	**1.54**	**0.026**	**2.10**	**0.002**	273, 274
7	Bb-regulatory subunit of protein phosphatase 2a	**5.40**	**0.001**	**4.26**	**0.002**	275, 276, 277, 278, 279
8	Chemokine receptor 4 (CXCR4)	2.18	≤ 0.100	2.03	≤ 0.100	280
9	Collagen alpha 1	**1.28**	**0.031**	**1.58**	**0.003**	281
10	Corticotropin releasing hormone (CRH)	**1.80**	**0.045**	**2.31**	**0.044**	282, 283

Table 4 (continuation): ***Influence of selenate at two different dietary levels (0.2 and 1.0 mg Se/kg diet) on the expression of genes critical for the development of insulin resistant diabetes and obesity compared to short term Se deficiency***

No.	Regulated gene	x-fold regulation 0.2 Selenate : 0Se	Error probability (t - Test)	x-fold regulation 1.0 Selenate : 0Se	Error probability (t - Test)	Literature
	Glutathione peroxidase 1	6.62	0.000	5.88	0.000	
	Phospholipid hydrop. glutathione peroxidase 4	1.32	0.014	1.31	0.016	
	Selenoprotein P	2.11	0.000	1.94	0.000	
11	Corticotropin releasing factor binding protein (CRHB)	**0.61**	**0.015**	**0.64**	**0.019**	282, 283
12	C-reactive protein (CRP)	1.37	0.015	1.33	0.001	284, 285
13	Dual specificity protein tyrosine phosphatase6 (MAPK3)	**0.52**	**0.002**	**0.50**	**0.001**	286
14	Fatty acid binding protein 1 (Liver FABP 1)	**1.27**	**0.000**	**1.69**	**0.000**	287
15	Fructose-1.6-bisphosphatase 1	**1.82**	**0.002**	**1.37**	**0.006**	288
16	Fructose-6-phosphate-2-kinase	**3.41**	**0.012**	**4.43**	**0.005**	289
17	Glucose-6-phosphatase	**2.21**	**0.000**	**2.28**	**0.001**	290
18	Glycogen phosphorylase	**2.06**	**≤ 0.100**	**2.76**	**0.046**	291, 292, 293, 294
19	Heme oxygenase-3 (HO 3)	**0.39**	**0.000**	**0.30**	**0.000**	295, 296, 297
20	Hypocretin orexin neuropeptide precursor (Prepro-orexin)	1.42	0.004	1.26	0.008	298, 299, 300, 301

Table 4 (continuation): ***Influence of selenate at two different dietary levels (0.2 and 1.0 mg Se/kg diet) on the expression of genes critical for the development of insulin resistant diabetes and obesity compared to short term Se deficiency***

No.	Regulated gene	x-fold regulation 0.2 Selenate : 0Se	Error probability (t - Test)	x-fold regulation 1.0 Selenate : 0Se	Error probability (t - Test)	Literature
	Glutathione peroxidase 1	6.62	0.000	5.88	0.000	
	Phospholipid hydrop. glutathione peroxidase 4	1.32	0.014	1.31	0.016	
	Selenoprotein P	2.11	0.000	1.94	0.000	
21	Hypocretin orexin receptor 1 (HCRTR 1)	**3.32**	**0.008**	**3.36**	**0.008**	302
22	Inducible prostaglandin E synthase	**3.33**	**0.023**	**4.58**	**0.001**	303
23	Insulin-like growth factor binding protein 1 (IGFBP 1)	**1.66**	**0.001**	**2.59**	**0.000**	304
24	Insulin-like growth factor binding protein 6 (IGFBP 6)	**2.16**	**0.032**	**2.65**	**0.023**	305
25	Interferon gamma receptor (IFNGR)	**1.54**	**0.045**	**1.43**	**≤ 0.100**	306
26	Interleukin 1 beta (IL1β)	**2.72**	**0.013**	**3.66**	**0.004**	307
27	Interleukin 10 (IL 10)	**0.37**	**0.010**	**0.41**	**0.012**	308, 309
28	Interleukin 10 receptor	**2.81**	**0.010**	**2.84**	**0.009**	310, 311, 312
29	Janus kinase 3 (JAK3)	**2.74**	**0.016**	**2.63**	**0.009**	313
30	Malic enzyme 1 (ME 1)	**0.66**	**0.004**	**0.66**	**0.004**	314, 315, 316

Table 4 (continuation): ***Influence of selenate at two different dietary levels (0.2 and 1.0 mg Se/kg diet) on the expression of genes critical for the development of insulin resistant diabetes and obesity compared to short term Se deficiency***

No.	Regulated gene	x-fold regulation 0.2 Selenate : 0Se	Error probability (t - Test)	x-fold regulation 1.0 Selenate : 0Se	Error probability (t - Test)	Literature
	Glutathione peroxidase 1	6.62	0.000	5.88	0.000	
	Phospholipid hydrop. glutathione peroxidase 4	1.32	0.014	1.31	0.016	
	Selenoprotein P	2.11	0.000	1.94	0.000	
31	MAP kinase phosphatase 2 (MKP 2)	**1.54**	**0.007**	**1.26**	**0.024**	317, 318, 319, 320, 321
32	MAP kinase phosphatase x (MKP x)	1.28	0.049	1.37	0.026	317, 318, 319, 320, 321
33	N-ethylmaleimide sensitive factor (ERG 1. NSF)	**0.57**	**0.009**	**0.39**	**0.002**	322, 323
34	Neuropeptide Y precursor (NPY)	**3.30**	**0.004**	**3.70**	**0.002**	299, 300, 324, 325
35	Neuropeptide Y 5 receptor (NPY 5 R)	1.61	$\leq$ 0.100	1.78	$\leq$ 0.100	299, 300, 324, 325
36	Ob-receptor gene related protein Ob-RGRP)	**3.00**	**0.046**	**3.96**	**0.019**	326
37	P 58 (DNAJC 3)	**2.85**	**0.008**	**2.09**	**0.037**	327
38	Pancreatic GLP1 receptor	1.32	0.008	1.31	0.009	328
39	Pancreatic lipase related protein 1 (PNLIPRP 1)	**2.84**	**0.039**	**2.75**	**0.049**	329, 330
40	Pancreatic serine threoine kinase (Ste-20)	**0.63**	**0.014**	**0.48**	**0.005**	331

Table 4 (continuation): ***Influence of selenate at two different dietary levels (0.2 and 1.0 mg Se/kg diet) on the expression of genes critical for the development of insulin resistant diabetes and obesity compared to short term Se deficiency***

No.	Regulated gene	x-fold regulation 0.2 Selenate : 0Se	Error probability (t - Test)	x-fold regulation 1.0 Selenate : 0Se	Error probability (t - Test)	Literature
	Glutathione peroxidase 1	6.62	0.000	5.88	0.000	
	Phospholipid hydrop. glutathione peroxidase 4	1.32	0.014	1.31	0.016	
	Selenoprotein P	2.11	0.000	1.94	0.000	
41	Phosphoenolpyruvate carboxykinase	**1.55**	**0.004**	**1.70**	**0.021**	332, 333, 334
42	Phosphorylase b kinase	**3.60**	**0.027**	**5.48**	**0.005**	293, 294, 295
43	Protein tyrosine phosphatase epsilon c (PTPεc)	**4.20**	**0.033**	**4.10**	**0.007**	335
44	Protein-tyrosine phosphatase 1B (PTP 1B)	**3.14**	**0.037**	**2.53**	**0.012**	336, 337
45	Pyruvate carboxylase	**1.48**	**0.025**	**1.73**	**0.005**	338
46	L-Pyruvate kinase	**0.42**	**0.011**	**0.76**	**0.050**	339
47	Squalene epoxidase (SQLE)	**1.95**	**0.047**	**2.12**	**0.021**	287
48	Synaptotagmin 8	**1.85**	**0.004**	**1.43**	**0.009**	340
49	Tgf-beta 2 long form precursor (TGF-b-2)	**1.85**	**0.004**	**1.31**	**0.016**	341, 342
50	Transcription factor FKHR (FOXO1a)	**2.79**	**0.005**	**1.27**	**0.031**	343, 344
51	Type 2 angiotensin receptor (AT2R)	**3.44**	**0.018**	**3.82**	**0.004**	345, 346
52	Vascular endothelial growth factor c (VEGFC)	**1.41**	**≤ 0.100**	**2.91**	**0.029**	347, 348

As mentioned above the aim of the second trial with growing rats consequently was to examine if PTP1B may be one part of the puzzle explaining undesirable effects of Se in the development of insulin resistant diabetes and obesity. Due to the fact that a distinct up-regulation of PTP1B mRNA was found in microarray analysis and that GPx1 participates in the regulation of cellular H_2O_2- and glutathione-levels, it could be assumed that PTP1B may be one promising molecular target explaining undesirable effects of dispensable Se on metabolic disorders.

In the second rat trial a distinct loss of GPx1 and GPx3 activity was measured in short term Se deficient rats (0Se) compared to their companions supplied with selenite and selenate at the recommended dietary level (0.2 mg Se/kg diet) and at two supranutritional levels (1.0 and 2.0 mg Se/kg diet). These results reflect the powerful effect of a lack in dietary Se supply on GPx1 and GPx3 activity, as intended by the experimental design. Simultaneously the low rank of these selenoproteins could be confirmed [37, 55]. That a dietary concentration of 0.2 mg Se/kg diet already meets the requirements for an abundant selenoprotein synthesis was also in agreement with literature [349].

- The first central finding of the second rat trial was the highly positive correlation that existed between dispensable Se supply and the activity of insulin antagonistic PTPs [258].

Supranutritional supply with both Se compounds (selenite and selenate) led to a dose dependent Se accumulation in liver and plasma, and it did not result in additional selenoprotein synthesis, but instead it caused a dose dependent increase in liver PTP activity [Figure 8]. The coherence between dispensable Se and PTP activity could be depicted by means of linear regression and square regression for liver (Figure 8 A, B) and plasma (Figure 8 C, D), respectively.

Since a clear coherence between a high Se status and the activity of the insulin antagonistic PTP could be pointed out in the second rat trial (Figure 8) the data may be helpful for further analysis of recent human studies showing a correlation between serum Se and diabetes incidence [264, 265].

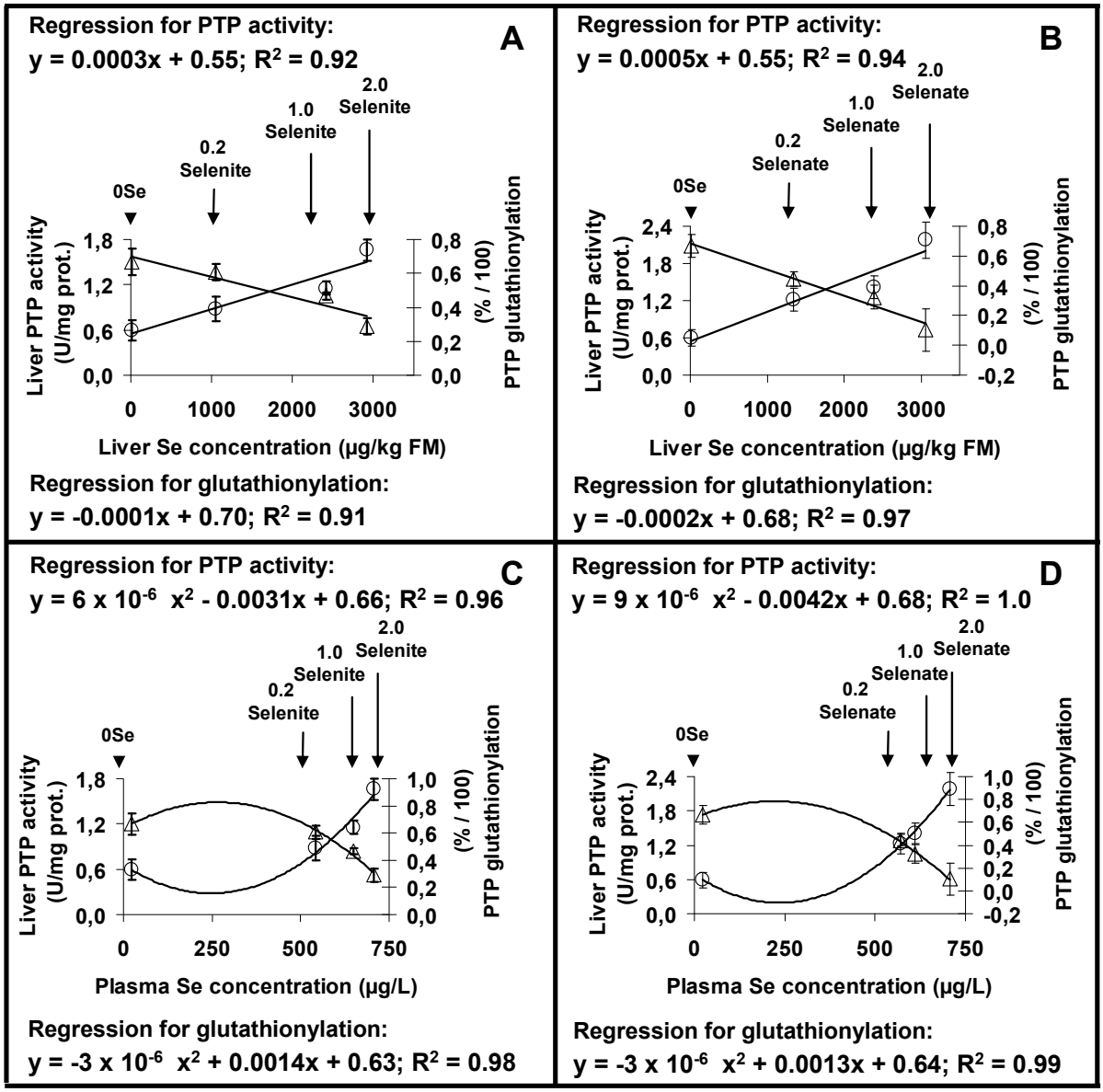


Figure 8: ***Regression analyses between liver Se concentration (A, B) or plasma Se concentration (C, D) and native liver PTP activity and PTP glutathionylation due to increasing Se supplementation as selenite (Se IV) [A, C] or selenate (Se VI) [B, D]***

- The second central finding consisted in the comprehension of the molecular mechanism by which GPx1 and supranutritional Se supplements modulate PTP1B activity [258]

In contrast to the mouse trial in which GPx1 overexpression promoted the development of obesity and insulin resistance [219, 220] the physiological approach of the second rat trial provides explanations by which Se supplements, exceeding the needs, can accelerate these diseases besides a high GPx1 activity. In the mouse trial a decreased tyrosine phosphorylation of the β subunit of the insulin receptor and a decreased phosphorylation of the downstream signalling protein Akt at Thr 308 and Ser 473 indicated the increased insulin resistance due to GPx1 overexpression [219, 220]. The data of the current rat trial however suggest that the decreased phosphorylation measured in the above mentioned trial more likely reflects an influence of the manipulated Se- and glutathione-dependent redox system on PTP1B than displaying a direct effect of GPx1 on protein phosphorylation. According to the results of the data from the current rat trial and to Figure 9 a lower dietary Se concentration and the resulting higher peroxide concentration due to a lack of GPx1 activity lead to a higher PTP1B inactivation by glutathionylation. Optimised activities of GPx1 by dietary Se (present study) [258] or an increase in GPx1 expression (mouse study) [219, 220] however remove H_2O_2 and disable PTP1B inhibition through glutathionylation [237-240, 242, 243]. These aspects therefore provide a plausible explanation for the development of insulin resistance and obesity due to a high GPx1 expression and activity via nutritional Se manipulation. Data of an above mentioned human study support this hypothesis by the finding that a high erythrocyte GPx1 corresponded to an increased incidence of gestational diabetes [221]. An up-regulation of PTP1B expression and with this an increase in intrinsic insulin resistance could also be found for mice overexpressing catalase, the second central enzyme in H_2O_2 detoxification [222]. Both the data of the first rat trial (microarray analysis and RT-PCR) as well as the data of the second rat trial could confirm an up-regulation of PTP1B mRNA expression through a high expression of GPx1 [257, 258] as another H_2O_2 detoxifying enzyme [Fig.1]. In physiological models, like the second rat trial, no overexpression of GPx1 could be obtained by increasing dietary Se concentration beyond the needs. Instead through this physiological model a way of PTP1B regulation, manipulating GPx1 expression and activity via a short term Se deficiency could be displayed.

Besides PTP1B regulation by H_2O_2 via GPx1 activity in the second rat trial two further important aspects of PTP1B regulation were found:

1. As discussed above the height of dispensable Se supply strongly influenced PTP activity.
2. Selenate (Se oxidation state +VI) led to a distinctly faster rise in PTP activity and corresponded to a stronger loss of PTP glutathionylation, compared to selenite.

These aspects of PTP regulation can be seen from the slopes of linear regression analyses (Figure 8 A, B) and could be visualized by Western Blot analysis [258]. As likewise found for the antidiabetic properties of high supranutritional selenate doses the latter aspect regarding a higher influence of selenate on PTP1B regulation presumably derives from fundamental differences in mammalian Se metabolism (Figure 9). The following physiological model seems to be plausible: Se from selenite (+IV) and selenate (+VI) is absorbed by individual mechanisms [13]. Selenite reacts with thiols like glutathione prior to its absorption and enters the peripheral organs in the form of selenotrisulfides (oxidation state: - I) or it is reduced in the erythrocytes to the selenide oxidation state –II and delivered to peripheral organs bound to albumin [15, 16]. In contrast unmodified selenate can be detected in the bloodstream and in peripheral tissues [18, 19, 20]. During successive selenate reduction the thiol reactive oxidation states (selenite: +IV, and selenotrisulfides: –I) can be formed and require glutathione for their further reduction to the selenide oxidation state (-II).

- Thus glutathione detraction from glutathionylated proteins could be one target for glutathione acquirement (Figure 9). Moreover our data suggest that *in vitro* effects of selenite (+IV) match selenate (+VI) feeding.

This particular aspect of mammalian Se metabolism could be visualized using an *in vitro* assay (Figure 10). Incubation of liver cytosol from group 0Se with increasing selenite or selenate concentrations, representing approximately the Se concentrations in the livers of rats receiving diets with 1.0 and 2.0 mg Se/kg, showed that unreactive selenate (+VI) did not influence PTP1B glutathionylation. In contrast selenite (+IV), matching selenate feeding, effected a dose-dependent loss of PTP1B glutathionylation.

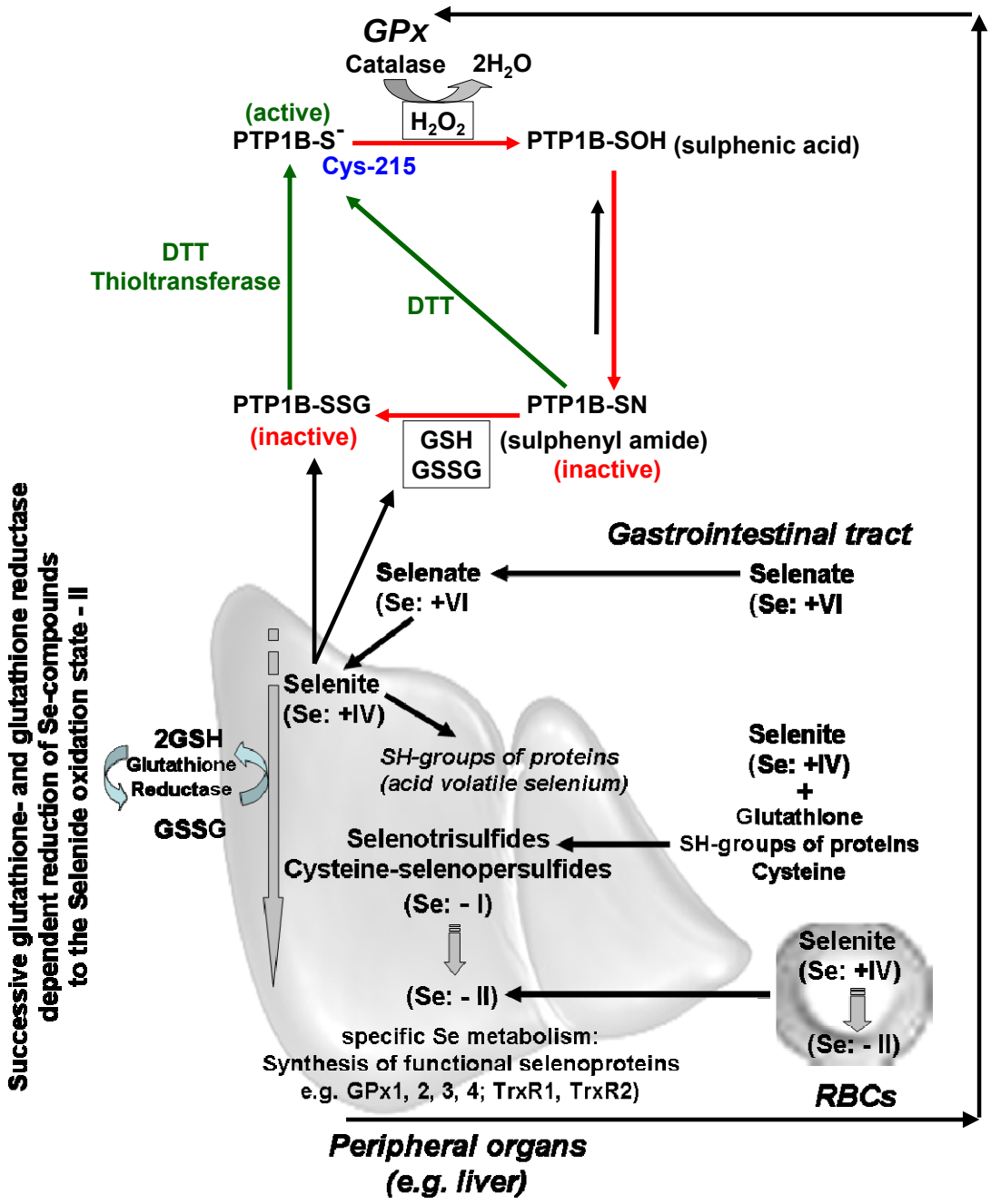


Figure 9: Current understanding of physiological PTP1B regulation and interfaces with mammalian Se metabolism

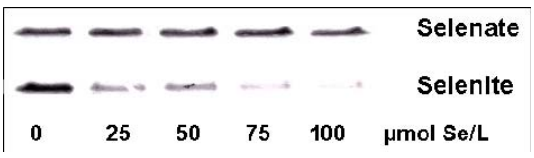


Figure 10: Glutathionylation of PTP1B after in vitro incubation of liver cytosol with increasing selenite or selenate concentrations – demonstrating that selenate feeding matches in vitro effects of selenite

A manipulation of PTP1B activity causes changes in a number of physiological parameters. In a mouse trial it could be shown that PTP1B deficient mice had a significantly higher energy expenditure than WT mice [232]. Despite a reduced feed intake in groups 2.0 Selenite and 2.0 Selenate of our trial, possibly deriving from an impaired palatability of high Se diets [350], the feed conversion ratio (= g feed intake : g body weight gain) was however significantly better in all Se supplemented groups

3.71±0.05 : 1 (0.2 Selenite), 3.73±0.02 : 1 (0.2 Selenate),

3.71±0.03 : 1 (1.0 Selenite), 3.81±0.04 : 1 (1.0 Selenate),

3.80±0.06 : 1 (2.0 Selenite), 3.74±0.03 : 1 (2.0 Selenate)

compared to group 0Se (4.00±0.10 : 1). According to the above mentioned mouse trial [232] the lower feed conversion in group 0Se could be an indicator for a higher energy expenditure due to a reduced PTP1B activity. In human studies and in animal trials PTP1B was demonstrated as one factor increasing body weight gain and the development of obesity [230-233, 235]. GPx1 overexpressing mice showed a significantly higher body weight and body fat gain [219, 220] whereas mice with a selenoprotein P (SeP) knockout and consequential lack of peripheral GPx1 synthesis were emaciated [138]. Thus our physiological study was in line with both trials [138, 219, 220], demonstrating that dispensable Se supply and high GPx1 activities are involved in body weight and fat gain and PTP1B regulation may be one factor mediating these effects.

Results from two rat studies dealing with the influence of PTP1B [259] and Se [351] on fatty acid metabolism give an idea how dispensable Se may promote the development of insulin resistance and obesity coevally.

In the first of the above mentioned trials insulin resistance and obesity in rats was induced by feeding diets with a high fructose content. Fructose fed rats showed a 3-fold elevated expression and activity of PTP1B. Due to the fact that a high PTP1B activity per se increases insulin resistance and additionally induces triglyceride synthesis via protein phosphatase 2A (PP2A) and sterol regulatory element binding protein 1c (SREBP1c), the genesis of both insulin resistance and obesity could be attributed to an elevated PTP1B activity [259].

In the second of the trials mentioned feeding a diet sufficient in Se (0.3 mg Se/kg) to rats for 12 weeks yielded a distinctly higher body weight, significantly higher concentrations of total liver lipids, liver triglycerides and liver cholesterol compared with rats kept on a Se deficient diet [351]. The authors of this study did not suggest a molecular mechanism for their data, but from the results of the present studies it can be assumed that the up-regulation of PTP1B by a high GPx1 activity and dispensable Se contributes to an accelerated development of insulin resistance and obesity (Figure 11). The underlying molecular mechanism thereby seems to be similar to that involved in fructose feeding (Figure 11).

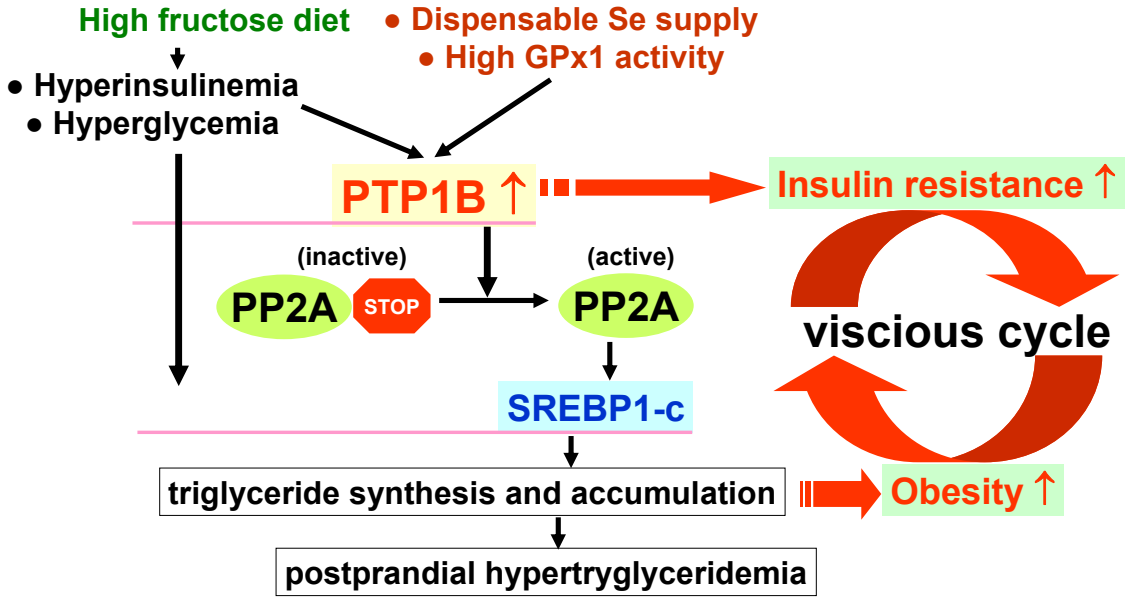


Figure 11: ***Molecular pathways by which nutrients increasing PTP1B activity can contribute to the accelerated development of insulin resistance and obesity***

This hypothesis is substantiated by the fact that in the present studies beside an up-regulation of PTP1B due to increasing Se supplementation also a strongly increased expression of the regulatory subunit of PP2A could be measured. Thus one likely molecular mechanism by which dispensable Se supplementation can accelerate the development of both insulin resistance and obesity involves triggering the expression and activity of PTP1B and PP2A, representing a mating gene assembly [259, 275-279, 335-337], as discussed above.

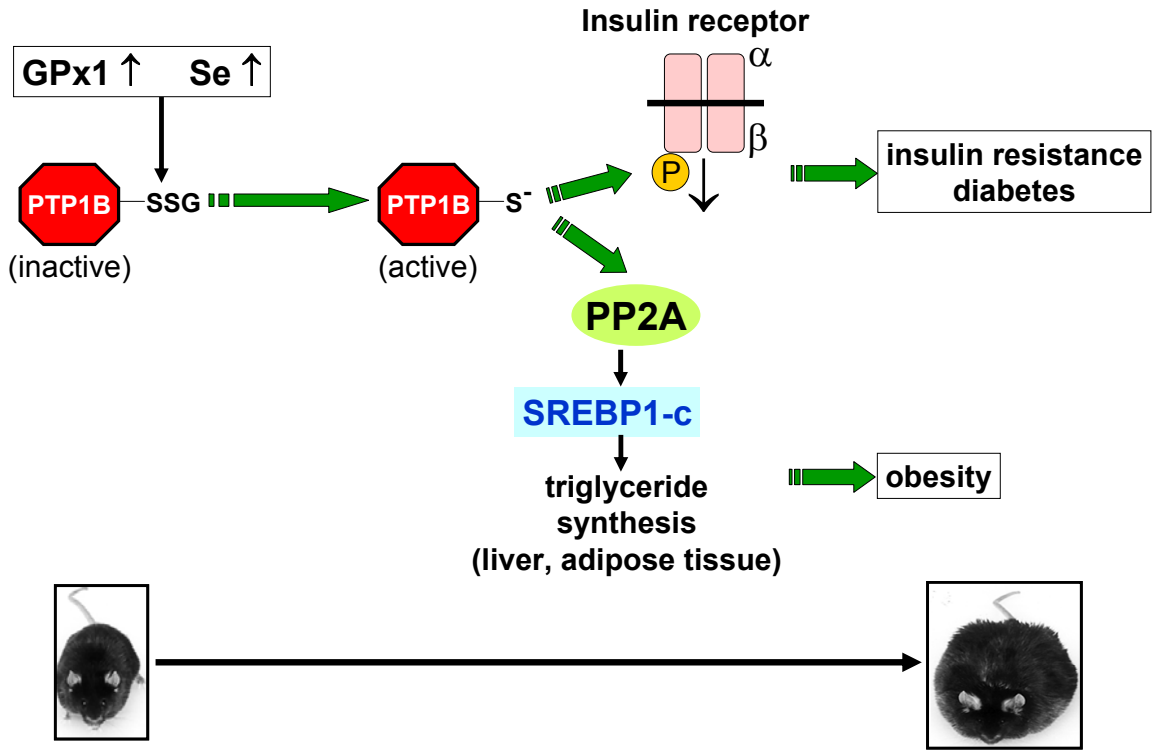


Figure 12: ***Molecular pathways by which Se can contribute to the accelerated development of insulin resistance and obesity***

A high GPx1 activity, as well as a high Se concentration in organs and plasma, leads to an activation of the insulin-antagonistic PTP1B which again can increase the risk for insulin resistance and obesity.

In conclusion the present studies could help in uncovering possible mechanisms by which a long term supranutritional Se supply may have undesirable effects on the development of insulin resistant diabetes and obesity: The regulation of PTP1B expression and activity by Se may be one part of this puzzle and the underlying molecular mechanism is summarized in the concluding Figure 12.

5.2.3 Future perspectives for research investigating the permanent supply of dispensable Se on an accelerated development of insulin resistant diabetes and obesity

Long term Se supply above the recommendations may be helpful in the therapy of prostate cancer [352] and with regard to some toxicological aspects where a decreased phosphorylation of critical signalling proteins due to Se supply is desirable [352, 353]. Concerning insulin resistance and obesity a retardation of phosphorylation signals via an increased PTP1B activity may be counterproductive and accelerate the development of

these diseases. In humans a saturated selenoprotein synthesis and therefore a sufficient antioxidant protection can be obtained by following the current recommendations (up to 70 µg Se/day, depending on age and physiological status) and result in serum plasma Se levels of 90 - 110 ng/mL. A permanent surplus of Se should not be recommended, since it will not redound to an increased biosynthesis of functional selenoproteins [354-356], but instead even accelerate the development of insulin resistant diabetes and obesity [219-221, 257, 258, 264, 265, 357-359].

This position was also defined in a “rapid response letter” to Annals of Internal Medicine [360] which is shown under 5.2.4.

Future studies in humans investigating the coherence between Se and diabetes should however include the examination of functional selenoproteins, of proteins involved in the development of insulin resistance and the state of the disease. Moreover future investigations focussing on the influence of Se in metabolic processes should consider interactions of Se with other antioxidants, with secondary plant substances, as well as the particular nutrition (carbohydrates, sugars, fat, fiber) in order to obtain a better risk assessment [361-363].

5.2.4 "Rapid response letter" to Annals of Internal Medicine

Annals of Internal Medicine

Selenium and diseases: Cancer or Diabetes? A few micrograms make a difference!

20 July 2007

Andreas S. Mueller,
PhD, nutrition physiology
Justus Liebig University Giessen, Department of Nutritional Physiology,
Astrid C. Bosse and Josef Pallauf

Send rapid response to journal:
Re: Selenium and diseases: Cancer or Diabetes? A few micrograms make a difference!

Email andreas.s.mueller@agrar.uni-giessen.de

Selenium and diseases: Cancer or Diabetes? A few micrograms make a difference! Presently a controversial and partially somewhat emotional discussion regarding benefits and disadvantages of selenium (Se) supplements for human health is in progress. Most recent data obtained from the NPC trial (1) as well as data from the NHANES III trial (2) however indicate a distinct correlation between a long-term high selenium status (measured by serum Se) and the incidence of diabetes in humans. We share the author's concluding remarks "that in populations with an adequate selenium supply an increased selenium intake should not be recommended for the prevention of diabetes. Quite the contrary long-term selenium supplementation above the current recommendations (50 – 70 µg Se daily) should be avoided because it may accelerate the development of obesity, insulin resistance and type II diabetes. This point of view can be substantiated on the basis of the facts from biochemical basic research on selenium:

1. Only in a very narrow therapeutic range selenium acts as an antioxidant. Antioxidant properties of selenium are only achieved by its biochemical functions in glutathione peroxidases and thioredoxin reductases whose maximum expression and activity is obtained already with relatively low selenium supply (3). The current recommendations (4) are absolutely sufficient for an optimum selenoprotein synthesis. A permanent surplus of selenium reverses its antioxidant effects, since selenium is a highly thiol reactive element.

2. Selenate (selenium oxidation state +VI) is the only selenium compound with proved antidiabetic effects (5, 6). An increased insulin signalling (phosphorylation) and can be obtained only with very high supranutritional doses through the intermediary formation of the selenium oxidation state +IV and the inhibition of insulin antagonistic protein tyrosine phosphatases. Selenate doses for the realization of antidiabetic effects are however by far too high for humans, and they were tested only in animal models and in tissue cultures so far (5, 6).

3. Both a permanent moderate surplus of selenium (7) as well as high activities of glutathione peroxidase (8) (shown by ~ 1.5-fold GPx1 overexpression in mice) can lead to a higher activity of the insulin antagonistic protein tyrosine phosphatase 1B (PTP1B) and therefore contribute to the development of obesity, insulin resistance and type II diabetes. In the mice study GPx1 overexpression reduced the β subunit of the insulin receptor as well as ofβphosphorylation of the downstream signalling protein Akt (at Thr308 and Ser473) indicating the insulin resistance (8).

4. Tragically one crucial mechanism by which a surplus of selenium may inhibit the progression of prostate cancer also involves Akt phosphorylation at Thr308 and Ser473 (9) With regard to the prevention of prostate cancer (10) a reduction of Akt

phosphorylation therefore is desirable (9). By its powerful influence on cellular signalling processes selenium is at the interface of two diseases (cancer and diabetes) which seem to be regulated in a contrary manner (Schema).

Level 1	Se↑	GPx1↑
Level 2	Protein phosphatases (e.g. PTEN, PTP1B)↑	
	Obesity, insulin resistance↑	
Level 3	Akt phosphorylation↓	
Level 4	Tumor progression↓	Glucose uptake↓
		Glycolysis↓
		Gluconeogenesis↑

Schema: Interactions between selenium and cellular signalling processes influencing metabolic disorders (obesity, insulin resistance, diabetes) and cancer

In conclusion a permanent use of selenium supplements should be reconsidered well. The evidence that a few micrograms of selenium may determine the accelerated or slowed development of diabetes and/or cancer demonstrates the necessity for intensified research in this field. Until then selenium supplements should not be recommended in populations with a sufficient intake by common nutrition.

1. Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, Cappuccio FP, Ceriello A, Reid ME. Effects of Long-Term Selenium Supplementation on the Incidence of Type 2 Diabetes: A Randomized Trial. Ann Intern Med 2007;147:217-223

2. Bleys J, Navas-Acien A, Guallar E. Serum selenium and diabetes in U.S. adults. Diabetes Care 2007; 30: 829-834

3. Gromer S, Eubel JK, Lee BL, Jacob J: Human selenoproteins at a glance. Cell Mol Life Sci 62: 2414 – 2437, 2005

4. NIH (National Institute of Health): ods.od.nih.gov/factsheets/selenium.asp. (Updated: 08/01/2004)

5. Stapleton SR, Garlock GL, Foellmi-Adams L, Kletzien RF. Selenium: potent stimulator of tyrosyl phosphorylation and activator of MAP kinase. Biochim Biophys Acta 1997; 1355(3): 259-269

6. Mueller AS, Pallauf J. Compendium of the antidiabetic effects of supranutritional selenate doses. In vivo and in vitro investigations with type II diabetic db/db mice. J Nutr Biochem 2006; 17: 548-560

7. Mueller AS, Pallauf J. Regulatory mechanisms of selenium and its compounds on protein tyrosine phosphatase 1B. In: Anke M et al. (eds): 23rd Workshop Macro and Trace Elements, Friedrich Schiller University Jena, Schubert-Verlag Leipzig, 2006, ISBN 3-929526-85-9, p. 196 – 203

8. McClung JP, Roneker CA, Mu W, Lisk DJ, Langlais P, Liu F, Lei XG. Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. Proc Natl Acad Sci U S A 2004; 101: 8852-8857

9. Wu Y, Zu K, Warren MA, Wallace PK, Ip C. Delineating the mechanism by which selenium deactivates Akt in prostate cancer cells. Mol Cancer Ther 2006;5: 246-252

10. Vogt TM, Ziegler RG, Patterson BH, Graubard BI. Racial Differences in Serum Selenium Concentration: Analysis of US Population Data from the Third National Health and Nutrition Examination Survey. Am J Epidemiol 2007; 166: 280-88

6. Summary

At present a controversial discussion regarding benefits and risks of selenium (Se) supplements on insulin resistant diabetes and obesity is in progress.

When starting the present studies with type II diabetic dbdb mice insulinomimetic properties of selenate (Se +VI) had been reported only for type I diabetic animal models and for tissue cultures. An increased phosphorylation of single proteins of the insulin signalling pathway was hypothesized to mediate the antidiabetic effects of selenate.

- No information was available if selenate has insulinomimetic properties also in type II diabetic species with insulin resistance.
- Moreover information was lacking whether the antidiabetic potential is restricted to high supranutritional selenate doses or if other inorganic Se compounds like selenite (Se IV) also act as antidiabetic agents.

In recent literature however opposite effects of Se on the development of insulin resistance and obesity have been reported both for animal models and human populations with a permanently high Se supply. In this context a supraphysiological activity of the peroxide detoxifying selenoenzyme glutathione peroxidase 1 (GPx1) has been demonstrated as diminishing insulin signalling and increasing intrinsic insulin resistance.

- The few results published regarding this undesirable aspect of Se were obtained however either from a non-physiological animal model with an overexpression of the selenoprotein GPx1 or they represented a merely statistical data analysis.
- Moreover these investigations did not provide sufficient explanations on mechanisms by which Se may develop undesirable influences on insulin resistant diabetes and obesity.
- A physiological model examining the effects of a permanent moderate surplus of Se on molecular pathways linked to insulin resistant diabetes and obesity was lacking.

The aim of the present studies consequently was to examine molecular coherences and mechanisms behind both aspects of the effects of Se on metabolic processes in different rodent species.

Two animal trials of the present studies focussed on the examination of antidiabetic effects of high supranutritional selenate doses in dbdb mice, representing an animal model suffering from severe insulin resistant diabetes and obesity. In these experiments it was also tested if antidiabetic effects of Se are restricted to selenate. In both trials three experimental groups of dbdb mice were fed a Se deficient basal diet for eight weeks. In each trial two of those

groups received high supranutritional Se doses (15 – 35 % of their individual half lethal dose) as selenite (Se +IV) or selenate (Se +VI).

Two further animal trials of the present studies with healthy growing rats investigated undesirable effects of Se on the development of insulin resistant diabetes and obesity. In these experiments one group of healthy growing rats was fed a Se deficient diet for eight weeks. Six further groups received diets containing Se at the recommended dietary level (0.2 mg Se/kg diet) and at two moderately supranutritional levels (1.0 and 2.0 mg Se/kg diet) as sodium selenite or sodium selenate.

- The oral application of high supranutritional selenate doses also clearly produced antidiabetic effects in type II diabetic dbdb mice with insulin resistance.
- In contrast selenite application was inefficient for diabetes therapy in dbdb mice.
- In the present work novel findings on the particular molecular mechanism behind the antidiabetic effects of selenate could be pointed out from the fact that selenate treatment effected a distinct amelioration of insulin resistance, followed by changes in the expression and activity of glycolytic and gluconeogenic key enzymes.
- Prior studies with type I diabetic animals have linked an increased phosphorylation of downstream proteins in the insulin signalling pathway (β subunit of the insulin receptor, MAPK, ribosomal S6 kinase) to the antidiabetic virtues of selenate. In the present work it could demonstrated that an increased phosphorylation of insulin signalling proteins and therefore antidiabetic virtues of selenate are based on the inhibition of protein tyrosine phosphatases (PTPs) rather than representing a direct influence of selenate on protein phosphorylation.
- In this context another original finding of the present work was that selenate's antidiabetic properties are keenly linked to mammalian Se metabolism. The results of an in vitro inhibition test for PTPs showed that PTP inhibition of orally applied selenate (Se +VI) derives from its intermediary reduction to the thiol-reactive selenite oxidation state +IV, acting as the actual PTP inhibitor. On this account it could be concluded that selenate feeding can be matched by the in vitro use of selenite.

- In the rat trials a number of candidate genes, which may mediate undesirable effects of Se on insulin resistant diabetes and obesity, could be evaluated by means of a microarray screening.
- In this screening the insulin antagonistic protein tyrosine phosphatase 1B (PTP1B) thereby represented one of the promising genes.

- The detailed study of PTP1B regulation revealed that the enzyme was up-regulated by both an optimization of GPx1 activity and by dietary Se supply. Thus dispensable Se supplementation led to a dose-dependent increase in PTP1B activity. The results of the present rat studies therefore could point out for the first time a plausible physiological mechanism by which a permanent surplus of Se can promote the development of insulin resistant diabetes and obesity.

In conclusion the present studies could contribute to a considerably better understanding of the molecular mechanisms by which high supranutritional selenate doses attain antidiabetic effects concerning insulin resistant type II diabetes. The safety of high supranutritional selenate doses, as required for antidiabetic effects, however has so far only been tested in tissue cultures and in animal models, and it is still questionable if selenate application in therapeutical and thus non-physiological doses can be relevant for diabetes treatment in humans.

Secondly the present studies with growing rats could find new aspects how the permanent use of Se supplements, moderately beyond the recommended amounts, may promote the development of insulin resistant diabetes and obesity. In conclusion these results demonstrate the need for future investigations focussing on the influence of Se in metabolic processes. Those investigations should also consider interactions of Se with other antioxidants, with secondary plant substances, as well as the particular nutrition (carbohydrates, sugars, fat, fiber) in order to obtain a better risk assessment. Since the permanent uptake of Se supplements seems to involve risks strict compliance with the current recommendations in human and animal nutrition should be ensured.

7. Zusammenfassung

Derzeit wird eine kontroverse wissenschaftliche Diskussion über Nutzen und Risiken einer zusätzlichen Supplementierung mit Selen (Se) bezüglich ihres Einflusses auf Typ II-Diabetes und Fettleibigkeit geführt.

Zum Zeitpunkt des Beginns der vorliegenden Untersuchungen existierten nur einige wenige Erkenntnisse bezüglich einer antidiabetischen Wirkung von Selenat (Se+VI). Diese Untersuchungen beschränkten sich außerdem auf typ I-diabetische Tiermodelle und Zellkulturmodelle. Als Grund für die „insulinomimetische“ Wirkung von Selenat wurde eine Erhöhung der Phosphorylierung bestimmter Proteine der Insulinsignalkaskade vermutet.

- Keine Informationen existierten, bezüglich einer antidiabetischen Wirkung hoch-supranutritiver Selenatdosierungen in typ II-diabetischen Modellorganismen mit einer hohen Insulinresistenz.
- Weiterhin bestand ein Informationsdefizit darüber, ob das antidiabetische Potenzial von Se nur auf Selenat begrenzt ist, oder ob andere anorganische Se Verbindungen wie Selenit ebenfalls als antidiabetische Agenzien anzusehen sind.

Im Gegensatz zu diesen antidiabetischen Eigenschaften des Selens wurden in neuesten Tier- und Humanstudien auch mögliche negative Auswirkungen einer bedarfsüberschreitenden Se Supplementierung auf die Entwicklung von Typ II-Diabetes und Fettleibigkeit aufgezeigt. In diesem Zusammenhang konnte in einem der angesprochenen Tierexperimente gezeigt werden, dass eine hohe Aktivität des Selenoenzyms Glutathionperoxidase 1 (GPx1) zu einer Verminderung des Insulin-Phosphorylierungssignals führte und somit eine erhöhte intrinsische Insulinresistenz auslöste.

- Die wenigen Untersuchungen, welche sich mit diesen unerwünschten Eigenschaften des Selens beschäftigten, besaßen jedoch aus zwei Gründen bislang nur eine eingeschränkte Aussagekraft: Entweder resultierten die Daten aus unphysiologischen Tiermodellen, in denen die Überexpression der GPx1 eine hohe diätetische Selenzufuhr repräsentieren soll, oder die Ergebnisse basierten auf einer rein statistischen Datenauswertung.
- Darüber hinaus gaben die bisher vorliegenden Untersuchungen noch keine ausreichenden mechanistischen Erklärungen bezüglich unerwünschter Effekte des Selens im Bezug auf Diabetes und Fettleibigkeit.
- Studien mit physiologischen Erklärungsansätzen existierten bis dato nicht.

Die Ziele der vorliegenden Arbeit bestanden somit einerseits darin molekulare Wirkungsmechanismen hinter antidiabetischen Effekten hoher Selenatdosierungen in Bezug auf Typ II-Diabetes zu untersuchen und andererseits mögliche physiologische Erklärungsansätze für eine beschleunigte Entwicklung von Typ II-Diabetes und Fettleibigkeit durch eine permanente Einnahme von Selensupplementen zu finden.

Zwei Tierversuche der vorliegenden Arbeit dienten der Untersuchung der antidiabetischen Wirkung hoher Selenatdosierungen auf Typ II-Diabetes. Diese Versuche wurden mit dbdb Mäusen durchgeführt, welche ein geeignetes Tiermodell für einen ausgeprägten Typ II-Diabetes mit Insulinresistenz sowie für Fettleibigkeit repräsentieren. In beiden Versuchen wurden jeweils alle drei Versuchsgruppen für acht Wochen mit einer Se armen Diät gefüttert. Den Tieren zweier Versuchsgruppen wurden in beiden Versuchen täglich hoch-supranutritive Mengen an Selenit oder Selenat per Schlundsonde verabreicht. Die verabreichten Mengen betrugen dabei 15-35% der halbletalen Dosis (LD_{50}).

Zwei weitere Tierexperimente der vorliegenden Arbeit waren der Untersuchung unerwünschter Eigenschaften von Selen in Bezug auf die Entwicklung von Typ II-Diabetes und Fettleibigkeit gewidmet. In diesen Versuchen wurde jeweils eine Se arme Diät für acht Wochen an eine Gruppe von gesunden heranwachsenden Albinoratten verfüttert. Sechs weitere Versuchsgruppen erhielten eine vergleichbare Diät, die jedoch mit Selenit oder mit Selenat in Höhe der derzeitigen Empfehlung (0.2 mg Se/kg Diät) bzw. in moderat supranutritiven Dosierungen (1.0 und 2.0 mg Se/kg Diät) supplementiert war.

- Die orale Applikation hoch-supranutritiver Selenatdosierungen hatte auch bei typ II-diabetischen dbdb Mäusen eine deutliches antidiabetisches Potenzial.
- Die Verabreichung von Selenit hingegen zeigte keinen therapeutischen Effekt.
- Die vorliegende Arbeit konnte neue Erkenntnisse bezüglich des antidiabetischen Wirkungsmechanismus von Selenat aufzeigen. In früheren Studien an typ I-diabetischen Tiermodellen wurde postuliert, dass die insulinomimetische Wirkung des Selenats auf einer Erhöhung der Phosphorylierung bestimmter Proteine in der Insulinsignalkaskade (β Untereinheit des Insulinrezeptors, MAPK, ribosomale S6 Kinase) basiert. Im Gegensatz zu diesen Erkenntnissen konnte in der vorliegenden Arbeit aufgezeigt werden, dass die erhöhte Phosphorylierung von Insulinsignalproteinen die Folge einer Inhibierung insulin-antagonistischer Protein Tyrosin Phosphatasen (PTPs) darstellt und nicht auf einem direkten Einfluss des Selenats auf die Proteinphosphorylierung beruht.

- Darüber hinaus konnte in der vorliegenden Arbeit zum ersten Mal dargestellt werden, dass die antidiabetische Wirkung des Selenats eng mit dem Se Metabolismus von Säugetieren verknüpft ist. Durch einen in vitro Inhibierungstest für PTPs konnte gezeigt werden, dass die inhibierende Wirkung von oral verabreichtem Selenat auf der intermediären Bildung der thiol-reaktiven Selenitoxidationsstufe +IV beruht, welche eine stark inhibierende Wirkung gegenüber PTPs zeigt. Daraus konnte geschlossen werden, dass die orale Selenatverabreichung mit der in vitro Wirkung von Selenit vergleichbar ist.

- In den Rattenversuchen konnte eine große Anzahl von Kandidatengenen, welche für unerwünschte Wirkungen des Selens bezogen auf die Entwicklung von Typ II-Diabetes und Fettleibigkeit verantwortlich sein könnten, mittels eines Microarray Screenings ermittelt werden.
- Die insulin-antagonistische Protein Tyrosin Phosphatase 1B (PTP1B), die in der Literatur als ein herausragendes Zielgen im Zusammenhang mit der Entwicklung von Insulinresistenz und Fettleibigkeit beschrieben wird, stellte ein Gen dar, welches durch Se deutlich heraufreguliert wurde.
- Die detaillierte Untersuchung der PTP1B Regulation zeigte, dass die Enzymaktivität der PTP1B sowohl durch eine Optimierung der GPx1 Aktivität als auch durch die diätetische Selenzufuhr heraufreguliert wird. Eine über den Bedarf hinausgehende Selenversorgung zog eine dosisabhängige Zunahme der PTP1B Aktivität nach sich.
- Die Ergebnisse der vorliegenden Arbeit konnten somit über die Regulation der PTP1B zum ersten Mal eine plausible physiologische Erklärung für eine begünstigte Entwicklung von Typ II-Diabetes und Fettleibigkeit durch eine permanente bedarfsübersteigende Se-Supplementierung aufzeigen.

Zusammenfassend konnte die vorliegende Arbeit neue Beiträge zum besseren Verständnis der molekularen Mechanismen hinter der antidiabetischen Wirkung hoch-supranutritiver Selenatdosierungen leisten. Ob die Anwendung solch hoher Selenatdosen in der Diabetestherapie beim Menschen von Relevanz sein könnte, bleibt jedoch bislang fraglich, da bisherige Erkenntnisse nur in Tiermodellen und in Zellkulturen ermittelt wurden.
Bezüglich der permanenten Einnahme von bedarfsübersteigenden Se Mengen, konnte die vorliegende Arbeit eine plausible Erklärung für eine fördernde Wirkung von Se auf die Genese von Typ II-Diabetes und Fettsucht geben, welche auf der Regulierung der PTP1B beruht. Die Ergebnisse der vorliegenden Arbeit machen einen zukünftigen Forschungsbedarf hinsichtlich der differenzierten Wirkung von Antioxidanzien wie Se auf metabolische Prozesse des Intermediärstoffwechsels deutlich. Solche zukünftigen Untersuchungen sollten

auch die Interaktion von Se mit anderen Antioxidanzien, mit sekundären Pflanzeninhaltstoffen sowie mit der spezifischen Ernährungsweise (Kohlenhydrat-lastig, Zucker- und Fett-betont, Faserreichtum) berücksichtigen, um eine noch bessere Risikobewertung vornehmen zu können. Da nach dem derzeitigen Kenntnisstand die permanente Einnahme von Selensupplementen auch deutliche Risiken zu beinhalten scheint, sollte die Selenzufuhr sowohl in der Humanernährung als auch in der Tierernährung die derzeitigen Empfehlungen nicht wesentlich übersteigen.

8. Literature index

1 Birringer M, Pilawa S, Flohé L. Trends in selenium biochemistry. Nat Prod Rep. 2002; 19: 693-718

2 Falbe J, Regitz M (eds). Römpp Chemie Lexikon 9th edition, Band 5 1992; Georg Thieme Verlag, Stuttgart; 4105-4110

3 Combs GF, Combs S (eds). The role of selenium in nutrition 1986. Academic Press, Orlando. 4 – 5, 107-121

4 Ganther HE, Kraus RJ. Oxidation states of glutathione peroxidase. Methods Enzymol. 1984; 107: 593-602

5 NRC (National Research Council). Nutrient requirements of laboratory animals. 4th revised ed., National Academy Press Washington D.C. 1995

6 NRC (National Research Council). Nutrient requirements of poultry. 9th revised ed., National Academy Press Washington D.C. 1994

7 NRC (National Research Council). Nutrient requirements of sheep. 6th revised ed., National Academy Press Washington D.C. 1995

8 NRC (National Research Council). Nutrient requirements of swine. 10th revised ed., National Academy Press Washington D.C. 1998

9 NIH (National Institute of Health) (Updated: 08/012004) ods.od.nih.gov/factsheets/selenium.asp.

10 Finley JW. Bioavailability of selenium from foods. Nutr Rev. 2006 Mar; 64(3): 146-51

11 Gmoshinskii IV, Mazo VK. Mineral substance in human nutrition. Selenium: absorption and bioavailability. Vopr Pitan. 2006; 75(5): 15-21

12 Wolffram S, Berger B, Grenacher B, Scharrer E. Transport of selenoamino acids and their sulfur analogues across the intestinal brush border membrane of pigs. J Nutr. 1989; 119: 706-712

13 Wolffram S, Arduser F, Scharrer E. In vivo intestinal absorption of selenate and selenite by rats. J Nutr. 1985; 115: 454-459

14 Beilstein MA, Whanger PD. Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine. J Nutr. 1986a; 116: 1711-1719

15 Beilstein MA, Whanger PD. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. J Nutr. 1986b; 116: 1701-1710

16 Suzuki KT, Shiobara Y, Itoh M, Omichi M. Selective uptake of selenite by red blood cells. Analyst 1998; 123: 63-67

17 Shiobara Y, Ogra Y, Suzuki KT. Speciation of metabolites of selenate in rats by HPLC-ICP-MS. Analyst 1999; 124: 1237-1241

18 Suzuki KT, Ogra Y. Metabolic pathway for selenium in the body: speciation by HPLC-ICP MS with enriched Se. Food Addit Contam. 2002 Oct;19(10):974-83

19 Suzuki KT, Ohta Y, Suzuki N. Availibility and metabolism of (77)Se-methylseleninic acid compared simultaneously with those of three related selenocompounds. Toxicol Appl Pharmacol. 2006; 217: 51-62

20 Suzuki KT, Doi C, Suzuki N. Metabolism of 76Se-methylselenocysteine compared with that of 77Se-selenomethionine and 82Se-selenite. Toxicol Appl Pharmacol. 2006 Dec 1;217(2):185-195

21 Diplock AT, Caygill CPJ, Jeffery EH, Thomas C. The nature of acid volatile selenium in the liver of the male rat. Biochem J. 1973; 134: 283 – 293

22 Ganther HE, Kraus RJ. Identification of hydrogen selenide and other volatile selenols by derivatization with 1-fluoro-2,4-dinitrobenzene. Anal Biochem. 1984; 138: 396 – 403

23 Hassoun BS, Palmer IS, Dwivedi C. Selenium detoxification by methylation. Res Commun Mol Pathol Pharmacol. 1995; 90: 133-142

24 Gromer S, Gross JH. Methylseleninate is a substrate rather than an inhibitor of mammalian thioredoxin reductase. Implications for the antitumor effects of selenium. J Biol Chem. 2002 Mar 22; 277(12): 9701-6

25 Dumont E, Vanhaecke F, Cornelis R. Selenium speciation from food source to metabolites: a critical review. Anal Bioanal Chem. 2006; 385: 1304-1323.

26 Sunde RA, Evenson JK. Serine incorporation into the selenocysteine moiety of glutathione peroxidase. J Biol Chem. 1997; 262: 933-937

27 Park SI, Park JM, Chittum HS, Yang ES, Carlson BA, Lee BJ, Hatfield DL. Selenocysteine tRNAs as central components of selenoprotein biosynthesis in eukaryotes. Biomed Environ Sci. 1997; 10: 116-124

28 Imai H, Sumi D, Hanamoto A, Arai M, Sugiyama A. Molecular cloning and functional expression of cDNA for rat phospholipid hydroperoxide glutathione peroxidase: 3´-untranslated region of the gene is necessary for functional expression. J Biochem Tokyo. 1995; 118: 1061-1067

29 Walczak R, Hubert N, Carbon P, Krol A. Solution structure of SECIS, the mRNA element required for eukaryotic selenocysteine insertion - interaction studies with the SECIS-binding protein SBP. Biomed Environ Sci. 1997; 10: 177-181

30 Böck A, Forchhammer K, Heider J, Leinfelder W, Sawers G, Veprek B, Zinoni F. Selenocysteine : the 21st amino acid. Mol Microbiol. 1991; 5: 515-520
31 Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973; 179: 588–590
32 Flohé L, Günzler WA, Schock HH. Glutathione peroxidase: a selenoenzyme. FEBS Lett. 1973; 32: 132–134
33 Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN. Characterization of mammalian selenoproteomes. Science 2003; 300: 1439–1443
34 Hall L, Williams K, Perry AC, Frayne J, Jury JA. The majority of human glutathione peroxidase type 5 (GPX5) transcripts are incorrectly spliced: implications for the role of GPX5 in the male reproductive tract. Biochem J. 1998; 333 (Pt 1): 5–9
35 Utomo A, Jiang X, Furuta S, Yun J, Levin DS, Wang YC, Desai KV, Green JE, Chen PL, Lee WH. Identification of a novel putative non-selenocysteine containing phospholipid hydroperoxide glutathione peroxidase (NPGPx) essential for alleviating oxidative stress generated from polyunsaturated fatty acids in breast cancer cells. J Biol Chem. 2004; 279: 43522–43529
36 Aumann KD, Bedorf N, Brigelius-Flohé R, Schomburg D, Flohé L. Glutathione peroxidase revisited – simulation of the catalytic cycle by computer-assisted molecular modelling. Biomed Environ Sci. 1997; 10: 136–155
37 Brigelius-Flohé R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med. 1999; 27: 951–965
38 Mills GC. Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects haemoglobin from oxidative breakdown. J Biol Chem. 1957; 229: 189–197
39 Food and Nutrition Board, Institute of Medicine (2000) Selenium. In: Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. A Report of the Panel on Dietary Antioxidants and Related Compounds, vol. 1, pp. 284–324, National Academy Press, Washington, DC
40 Müller S, Riedel HD, Stremmel W. Direct evidence for catalase as the predominant H_2O_2 - removing enzyme in human erythrocytes. Blood 1997; 90: 4973–4978
41 Ho YS, Magnenat JL, Bronson RT, Cao J, Gargano M, Sugawara M, Funk CD. Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. J Biol Chem. 1997; 272: 16644–16651
42 Fu Y, Cheng WH, Porres JM, Ross DA, Lei XG. Knockout of cellular glutathione peroxidase gene renders mice susceptible to diquat-induced oxidative stress. Free Radic Biol Med. 1999; 27: 605–611
43 Cheng WH, Quimby FW, Lei XG. Impacts of glutathione peroxidase-1 knockout on the protection by injected selenium against the pro-oxidant-induced liver aponecrosis and signaling in selenium-deficient mice. Free Radic Biol Med. 2003 Apr 1; 34(7): 918-27
44 Cheng WH, Valentine BA, Lei XG. High levels of dietary vitamin E do not replace cellular glutathione peroxidase in protecting mice from acute oxidative stress. J Nutr. 1999 Nov; 129(11): 1951-7
45 Cheng WH, Ho YS, Valentine BA, Ross DA, Combs GF Jr, Lei XG. Cellular glutathione peroxidase is the mediator of body selenium to protect against paraquat lethality in transgenic mice. J Nutr. 1998 Jul; 128(7): 1070-6
46 Florian S, Wingler K, Schmehl K, Jacobasch G, Kreuzer OJ, Meyerhof W, Brigelius-Flohé R. Cellular and subcellular localization of gastrointestinal glutathione peroxidase in normal and malignant human intestinal tissue. Free Radic Res. 2001; 35: 655–663
47 Wingler K. and Brigelius-Flohé R. Gastrointestinal glutathione peroxidase. Biofactors 1999; 10: 245–249
48 Wingler K, Bocher M, Flohé L, Kollmus H, Brigelius-Flohé R. mRNA stability and selenocysteine insertion sequence effi ciency rank gastrointestinal glutathione peroxidase high in the hierarchy of selenoproteins. Eur J Biochem. 1999; 259: 149–157
49 Brigelius-Flohé R, Muller C, Menard J, Florian S, Schmehl K, Wingler K. Functions of GI-GPx: lessons from selenium-dependent expression and intracellular localization. Biofactors 2001; 14: 101–106
50 Chu FF, Esworthy RS, Chu PG, Longmate JA, Huycke MM, Wilczynski S, Doroshow JH. Bacteria-induced intestinal cancer in mice with disrupted Gpx1 and Gpx2 genes. Cancer Res. 2004; 64: 962–968
51 Takahashi K, Akasaka M, Yamamoto Y, Kobayashi C, Mizoguchi J, Koyama J. Primary structure of human plasma glutathione peroxidase deduced from cDNA sequences. J Biochem. (Tokyo) 1990; 108: 145–148
52 Tham DM, Whitin JC, Kim KK, Zhu SX, Cohen HJ. Expression of extracellular glutathione peroxidase in human and mouse gastrointestinal tract. Am J Physiol. 1998; 275: G1463–1471
53 Takahashi K, Avissar N, Whitin J, Cohen H. Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. Arch Biochem Biophys. 1987; 256: 677–686

54 Björnstedt M, Xue J, Huang W, Akesson B, Holmgren A. The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. J Biol Chem. 1994; 269: 29382–29384
55 Bierl C, Voetsch B, Jin RC, Handy DE, Loscalzo J. Determinants of human plasma glutathione peroxidase (GPx-3) expression. J Biol Chem 2004; 279: 26839–26845
56 Sarto C, Frutiger S, Cappellano F, Sanchez JC, Doro G, Catanzaro F, Hughes GJ, Hochstrasser DF, Mocarelli P. Modified expression of plasma glutathione peroxidase and manganese superoxide dismutase in human renal cell carcinoma. Electrophoresis 1999; 20: 3458–3466
57 Whitin JC, Tham DM, Bhamre S, Ornt DB, Scandling JD, Tune BM, Salvatierra O, Avissar N, Cohen HJ. Plasma glutathione peroxidase and its relationship to renal proximal tubule function. Mol Genet Metab. 1998; 65: 238–245
58 Ursini F, Maiorino M, Valente M, Ferri L, Gregolin C. Purifi cation from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. Biochim Biophys Acta. 1982; 710: 197–211
59 Ursini F, Maiorino M, Gregolin C. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim Biophys Acta. 1985; 839: 62–70
60 Brigelius-Flohé R, Friedrichs B, Maurer S, Schultz M, Streicher R. Interleukin-1-induced nuclear factor kappa B activation is inhibited by overexpression of phospholipid hydroperoxide glutathione peroxidase in a human endothelial cell line. Biochem J. 1997; 328 (Pt 1): 199–203
61 Foresta C, Flohé L, Garolla A, Roveri A, Ursini F, Maiorino M. Male fertility is linked to the selenoprotein phospholipid hydroperoxide glutathione peroxidase. Biol Reprod. 2002; 67: 967–971
62 Flohé L, Foresta C, Garolla A, Maiorino M, Roveri A, Ursini F. Metamorphosis of the selenoprotein PHGPx during spermatogenesis. Ann NY Acad Sci. 2002; 973: 287–288
63 Moreno SG, Laux G, Brielmeier M, Bornkamm GW, Conrad M. Testis-specifi c expression of the nuclear form of phospholipid hydroperoxide glutathione peroxidase (PHGPx). Biol Chem. 2003; 384: 635–643
64 Ursini F, Heim S, Kiess M, Maiorino M, Roveri A, Wissing J, Flohé L. Dual function of the selenoprotein PHGPx during sperm maturation. Science 1999; 285: 1393–1396
65 Maiorino M, Bosello V, Ursini F, Foresta C, Garolla A, Scapin M, Sztajer H, Flohe L. Genetic variations of gpx-4 and male infertility in humans. Biol Reprod. 2003; 68: 1134–1141
66 Vogt PH. Molecular genetics of human male infertility: from genes to new therapeutic perspectives. Curr Pharm Des. 2004; 10: 471–500
67 Yant LJ, Ran Q, Rao L, Van Remmen H, Shibatani T, Belter JG, Motta L, Richardson A, Prolla TA. The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. Free Radic Biol Med. 2003; 34: 496–502
68 Okamura N, Iwaki Y, Hiramoto S, Tamba M, Bannai S, Sugita Y, Syntin P, Dacheux F, Dacheux JL. Molecular cloning and characterization of the epididymis-specific glutathione peroxidase-like protein secreted in the porcine epididymal fluid. Biochim Biophys Acta. 1997; 1336: 99–109
69 Vernet P, Rigaudiere N, Ghyselinck N, Dufaure JP, Drevet JR. In vitro expression of a mouse tissue specific glutathione-peroxidase-like protein lacking the selenocysteine can protect stably transfected mammalian cells against oxidative damage. Biochem Cell Biol. 1996; 74: 125–131
70 Papp LV, Lu J, Holmgren A, Khanna KK. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal. 2007; 9: 775-806
71 Williams CHJ. (1992) Lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase, and mercuric ion reductase – a family of fl avoenzyme transhydrogenases in: Chemistry and biochemistry of fl avoenzymes, vol. 3, pp. 121–211, Müller F. (ed.), CRC Press, Boca Raton, FL
72 Novoselov SV, Gladyshev VN. Non-animal origin of animal thioredoxin reductases: implications for selenocysteine evolution and evolution of protein function through carboxy-terminal extensions. Protein Sci. 2003; 12: 372–378
73 Tamura T, Stadtman TC. A new selenoprotein from human lung adenocarcinoma cells: purifi cation, properties and thioredoxin reductase activity. Proc Natl Acad Sci. USA 1996; 93: 1006–1011
74 Gromer S, Arscott LD, Williams CH Jr, Schirmer RH, Becker K. Human placenta thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics, and inhibition by therapeutic gold compounds. J Biol Chem. 1998; 273: 20096–20101
75 Gromer S, Schirmer RH, Becker K. The 58 kDa mouse selenoprotein is a BCNU-sensitive thioredoxin reductase. FEBS Lett. 1997; 412: 318–320

76 Gladyshev VN, Jeang KT, Stadtman TC. Selenocysteine, identified as the penultimate C-terminal residue in human T-cell thioredoxin reductase, corresponds to TGA in the human placental gene. Proc Natl Acad Sci. USA 1996; 93: 6146–6151
77 Sun QA, Zappacosta F, Factor VM, Wirth PJ, Hatfield DL, Gladyshev VN. Heterogeneity within animal thioredoxin reductases. Evidence for alternative first exon splicing. J Biol Chem. 2001; 276: 3106–3114
78 Rundlöf AK, Janard M, Miranda-Vizuete A, Arnér ES. Evidence for intriguingly complex transcription of human thioredoxin reductase 1. Free Radic Biol Med. 2004; 36: 641–656
79 Nonn L, Williams RR, Erickson RP, Powis G. The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. Mol Cell Biol. 2003; 23: 916–922
80 Matsui M, Oshima M, Oshima H, Takaku K, Maruyama T, Yodoi J, Taketo MM. Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. Dev Biol. 1996; 178: 179–185
81 Gromer S, Johansson L, Bauer H, Arscott LD, Rauch S, Ballou DP, Williams CH Jr, Schirmer RH, Arnér ES. Active sites of thioredoxin reductases: why selenoproteins? Proc Natl Acad Sci. USA 2003; 100: 12618–12623
82 Gromer S, Urig S, Becker K. The thioredoxin system-from science to clinic. Med Res Rev. 2004; 24: 40–89
83 Sun QA, Gladyshev VN. Redox regulation of cell signaling by thioredoxin reductases. Methods Enzymol. 2002; 347: 451–461
84 Anestal K, Arnér ESJ. Rapid induction of celldeath by selenium compromised thioredoxin reductase 1 but not by the fully active enzyme containing selenocysteine. J Biol Chem. 2003; 6: 6
85 Spyrou G, Holmgren A. Deoxyribonucleoside triphosphate pools and growth of glutathione-depleted 3T6 mouse fi broblasts. Biochem Biophys Res Commun. 1996; 220: 42–46
86 Söderberg A, Sahaf B, Rosén A. Thioredoxin reductase, a redox-active selenoprotein, is secreted by normal and neoplastic cells: presence in human plasma. Cancer Res. 2000; 60: 2281–2289
87 Miranda-Vizuete A, Damdimopoulos AE, Spyrou G. The mitochondrial thioredoxin system. Antioxid Redox Signal. 2000; 2: 801–810
88 Lescure A, Gautheret D, Carbon P, Krol A. Novel selenoproteins identifi ed in silico and in vivo by using a conserved RNA structural motif. J Biol Chem. 1999; 274: 38147–38154
89 Ge K, Xue A, Bai J, Wang S. Keshan disease-an endemic cardiomyopathy in China. Virchows Arch. A Pathol Anat Histopathol. 1983; 401: 1–15
90 Conrad M, Jakupoglu C, Moreno SG, Lippl S, Banjac A, Schneider M, Beck H, Hatzopoulos AK, Just U, Sinowatz F, Schmahl W, Chien KR, Wurst W, Bornkamm GW, Brielmeier M. Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. Mol Cell Biol. 2004; 24: 9414–9423
91 May JM, Mendiratta S, Hill KE, Burk RF. Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. J Biol Chem. 1997; 272: 22607-22610
92 Kumar S, Bjornstedt M, Holmgren A. Selenite is a substrate for calf thymus thioredoxin reductase and thioredoxin and elicits a large non-stoichiometric oxidation of NADPH in the presence of oxygen. Eur J Biochem. 1992 Jul 15; 207(2): 435-39
93 Sun QA, Kirnarsky L, Sherman S, Gladyshev VN. Selenoprotein oxidoreductase with specificity for thioredoxin and glutathione systems. Proc Natl Acad Sci. USA 2001; 98: 3673–3678
94 Behne D, Kyriakopoulos A, Meinhold H, Koehrle J. Identification of type I iodothyronine 5'-deiodinase as a selenoenzyme. Biochem Biophys Res Commun. 1990; 173: 1143–1149
95 Arthur JR, Nicol F, Beckett GJ. Hepatic iodothyronine 5'-deiodinase. The role of selenium. Biochem J. 1990; 272: 537–540
96 Dickson RC, Tomlinson RH. Selenium in blood and human tissues. Clin Chim Acta. 1967; 16: 311–321
97 Koehrle J. The trace element selenium and the thyroid gland. Biochimie 1991; 81: 527–533
98 Moreno-Reyes R, Suetens C, Mathieu F, Begaux F, Zhu D, Rivera MT, Boelaert M, Nève J, Perlmutter N, Vanderpas J. Kashin-Beck osteoarthropathy inrural Tibet in relation to selenium and iodine status. N Engl J Med. 1998; 339: 1112–1120
99 Contempre B, de Escobar GM, Denef JF, Dumont JE, Many MC. Thiocyanate induces cell necrosis and fibrosis in selenium- and iodine-defi cient rat thyroids: a potential experimental model for myxedematous endemic cretinism in central Africa. Endocrinology 2004; 145: 994–1002
100 Koehrle J. Local activation and inactivation of thyroid hormones: the deiodinase family. Mol Cell Endocrinol. 1999; 151: 103–119
101 Berry MJ, Banu L, Larsen PR. Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. Nature 1991; 349: 438–440

102 Berry MJ, Banu L, Chen YY, Mandel SJ, Kieffer JD, Harney JW, Larsen PR. Recognition of UGA as a selenocysteine codon in type I deiodinase requires sequences in the 3' untranslated region. Nature 1991; 353: 273–276

103 Kelly GS. Peripheral metabolism of thyroid hormones: a review. Altern Med Rev. 2000; 5: 306–333

104 Leonard JL, Rosenberg IN. Thyroxine 5'-deiodinase activity of rat kidney: observations on activation by thiols and inhibition by propylthiouracil. Endocrinology 1978; 103: 2137–2144

105 Chaudière J, Tappel AL. Interaction of gold(I) with the active site of selenium-glutathione peroxidase. J Inorg Biochem. 1984; 20: 313–325

106 Gromer S, Arscott LD, Williams CH Jr, Schirmer RH, Becker K. (1998) Human placenta thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics, and inhibition by therapeutic gold compounds. J Biol Chem. 1998; 273: 20096-20101.

107 Buettner C, Harney JW, Larsen PR. The 3'-untranslated region of human type 2 iodothyronine deiodinase mRNA contains a functional selenocysteine insertion sequence element. J Biol Chem. 1998; 273: 33374–33378

108 Araki O, Murakami M, Morimura T, Kamiya Y, Hosoi Y, Kamiya Y, Morimura T, Ogiwara T, Mizuma H, Mori M. Assignment of type II iodothyronine deiodinase gene (DIO2) to human chromosome band 14q24.2->q24.3 by in situ hybridization. Cytogenet Cell Genet. 1999; 84: 73–74

109 Escobar-Morreale HF, Obregon MJ, Escobar del Rey F, Morreale de Escobar G. Tissue-specific patterns of changes in 3,5,3'-triiodo-L-thyronine concentrations in thyroidectomised rats infused with increasing doses of the hormone. Which are the regulatory mechanisms? Biochimie 1999; 81: 453–462

110 Bartha T, Kim SW, Salvatore D, Gereben B, Tu HM, Harney JW, Rudas P, Larsen PR. Characterization of the 5'-fl anking and 5'-untranslated regions of the cyclic adenosine 3',5'-monophosphate-responsive human type 2 iodothyronine deiodinase gene. Endocrinology 2000; 141: 229–237

111 Schneider MJ, Fiering SN, Pallud SE, Parlow AF, St Germain DL, Galton VA. Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. Mol Endocrinol. 2001; 15: 2137–2148

112 Ng L, Goodyear RJ, Woods CA, Schneider MJ Schneider MJ, Diamond E, Richardson GP, Kelley MW, Germain DL, Galton VA, Forrest D. Hearing loss and retarded cochlear development in mice lacking type 2 iodothyronine deiodinase. Proc Natl Acad Sci. USA 2004; 101: 3474–3479

113 Salvatore D, Low SC, Berry M, Maia AL, Harney JW, Croteau W, St Germain DL, Larsen PR. Type 3 lodothyronine deiodinase: cloning, in vitro expression, and functional analysis of the placental selenoenzyme. J Clin Invest. 1995; 96: 2421–2430

114 Mortimer RH, Galligan JP, Cannell GR, Addison RS, Roberts MS. Maternal to fetal thyroxine transmission in the human term placenta is limited by inner ring deiodination. J Clin Endocrinol Metab. 1996; 81: 2247–2249

115 Mostert V. Selenoprotein P: properties, functions and regulation. Arch Biochem Biophys. 2000; 376: 433–438

116 Motsenbocker MA, Tappel AL. A selenocysteine-containing selenium-transport protein in rat plasma. Biochim Biophys. 1982; Acta 719: 147–153

117 Eberle B, Haas HJ. Purifi cation of selenoprotein P from human plasma. J Trace Elem Electrolytes Health Dis. 1993; 7: 217–221

118 Schweizer U, Streckfuss F, Pelt P, Carlson BA, Hatfield DL, Koehrle J, Schomburg L. Hepatically derived selenoprotein P is a key factor for kidney but not for brain selenium supply. Biochem J. 2005; 386: 221–226

119 Gomez B Jr, Tappel AL. Selenoprotein P receptor from rat. Biochim Biophys. 1989; Acta 979: 20–26

120 Hill KE, Lloyd RS, Yang JG, Read R, Burk RF. The cDNA for rat selenoprotein P contains 10 TGA codons in the open reading frame. J Biol Chem. 1991; 266: 10050–10053

121 Burk RF, Hill KE. Some properties of selenoprotein P. Biol Trace Elem Res. 1992; 33: 151–153

122 Motchnik PA, Tappel AL. Multiple selenocysteine content of selenoprotein P in rats. J Inorg Biochem. 1990; 40: 265–269

123 Ma S, Hill KE, Burk RF, Caprioli RM. Mass spectrometric determination of selenenylsulfi de linkages in rat selenoprotein P. J Mass Spectrom. 2005; 40: 400–404

124 Persson-Moschos M, Huang W, Srikumar TS, Akesson B, Lindeberg S. Selenoprotein P in serum as a biochemical marker of selenium status. Analyst 1995; 120: 833–836

125 Dreher I, Jakobs TC, Koehrle J. Cloning and characterization of the human selenoprotein P promoter. Response of selenoprotein P expression to cytokines in liver cells. J Biol Chem. 1997; 272: 29364–29371

126 Saito Y, Takahashi K. Characterization of selenoprotein P as a selenium supply protein. Eur J Biochem. 2002; 269: 5746–51

127 Saito Y, Sato N, Hirashima M, Takebe G, Nagasawa S, Takahashi K. Domain structure of bi-functional selenoprotein P. Biochem J. 2004; 381: 841–846
128 Burk RF, Hill KE, Read R, Bellew T. Response of rat selenoprotein P to selenium administration and fate of its selenium. Am J Physiol. 1991; 261: E26–30
129 Kaim W, Schwederski B. (1995) Bioanorganische Chemie: zur Funktion chemischer Elemente in Lebensprozessen, p. 460, Teubner, Stuttgart
130 Yoneda S, Suzuki KT. Equimolar Hg-Se complex binds to selenoprotein P. Biochem Biophys Res Commun. 1997; 231: 7–11
131 Suzuki KT, Sasakura C, Yoneda S. Binding sites for the (Hg-Se) complex on selenoprotein P. Biochim Biophys. 1998; Acta 1429: 102–112
132 Fujii M, Saijoh K, Sumino K. Regulation of selenoprotein P mRNA expression in comparison with metallothionein and osteonectin mRNAs following cadmium and dexamethasone administration. Kobe J Med Sci. 1997; 43: 13–23
133 Fujii M, Saijoh K, Kobayashi T, Fujii S, Lee MJ, Sumino K. Analysis of bovine selenoprotein P-like protein gene and availability of metal responsive element (MRE) located in its promoter. Gene 1997; 199: 211–217
134 Saito Y, Hayashi T, Tanaka A, Watanabe Y, Suzuki M, Saito E, Takahashi K. Selenoprotein P in human plasma as an extracellular phospholipid hydroperoxide glutathione peroxidase. Isolation and enzymatic characterization of human selenoprotein P. J Biol Chem. 1999; 274: 2866–2871
135 Arteel GE, Mostert V, Oubrahim H, Briviba K, Abel J, Sies H. Protection by selenoprotein P in human plasma against peroxynitrite-mediated oxidation and nitration. Biol Chem. 1998; 379: 1201–1205
136 Schweizer U., Schomburg L. and Savaskan N. E. (2004) The neurobiology of selenium: lessons from transgenic mice. J. Nutr. 134: 707–710
137 Hill KE, Zhou J, McMahan WJ, Motley AK, Atkins JF, Gesteland RF, Burk RF. Deletion of selenoprotein P alters distribution of selenium in the mouse. J Biol Chem. 2003; 278: 13640–13646
138 Schomburg L, Schweizer U, Holtmann B, Flohé L, Sendtner M, Koehrle J. Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues. Biochem J. 2003; 370: 397–402
139 Bösl MR, Takaku K, Oshima M, Nishimura S, Taketo MM. Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp). Proc Natl Acad Sci. USA 1997; 94: 5531–5534
140 Schweizer U, Michaelis M, Koehrle J, Schomburg L. Efficient selenium transfer from mother to offspring in selenoprotein P-deficient mice enables dose-dependent rescue of phenotypes associated with selenium deficiency. Biochem J. 2004; 378: 21–26
141 Olson GE, Winfrey VP, Nagdas SK, Hill KE, Burk RF. Selenoprotein P is required for mouse sperm development. Biol Reprod. 2005; 73: 201–211
142 Richardson DR. More roles for selenoprotein P: local selenium storage and recycling protein in the brain. Biochem J. 2005; 386: e5–7
143 Calvo A, Xiao N, Kang J, Best CJ, Leiva I, Emmert-Buck MR, Jorcyk C, Green JE. Alterations in gene expression profi les during prostate cancer progression: functional correlations to tumorigenicity and down-regulation of selenoprotein-P in mouse and human tumors. Cancer Res. 2002; 62: 5325–5335
144 Zachara BA, Szewczyk-Golec K, Tyloch J, Wolski Z, Szylberg T, Stepien S, Kwiatkowski S, Bloch-Boguslawska E, Wasowicz W. Blood and tissue selenium concentrations and glutathione peroxidase activities in patients with prostate cancer and benign prostate hyperplasia. Neoplasma 2005; 52: 248–254
145 Zachara BA, Szewczyk-Golec K, Wolski Z, Tyloch J, Skok Z, Bloch-Boguslawska E, Wasowicz W. Selenium level in benign and cancerous prostate. Biol. Trace Elem Res. 2005; 103: 199–206
146 Low SC, Harney JW, Berry MJ. Cloning and functional characterization of human selenophosphate synthetase, an essential component of selenoprotein synthesis. J Biol Chem. 1995; 270: 21659–21664
147 Guimarães MJ, Peterson D, Vicari A, Cocks BG, Copeland NG, Gilbert DJ, Jenkins NA, Ferrick DA, Kastelein RA, Bazan JF, Zlotnik A. Identification of a novel selD homolog from eukaryotes, bacteria, and archaea: is there an autoregulatory mechanism in selenocysteine metabolism? Proc Natl Acad Sci. USA 1996; 93: 15086–15091
148 Lacourciere GM, Stadtman TC. Utilization of selenocysteine as a source of selenium for selenophosphate biosynthesis. Biofactors 2001; 14: 69–74
149 Tamura T, Yamamoto S, Takahata M, Sakaguchi H, Tanaka H, Stadtman TC, Inagaki K. Selenophosphate synthetase genes from lung adenocarcinoma cells: Sps1 for recycling Lselenocysteine and Sps2 for selenite assimilation. Proc Natl Acad Sci. USA 2004; 101: 16162–16167

150 Ogasawara Y, Lacourciere GM, Ishii K, Stadtman TC. Characterization of potential selenium-binding proteins in the selenophosphate synthetase system. Proc Natl Acad Sci. USA 2005; 102: 1012–1016
151 Lacourciere GM. Selenium is mobilized in vivo from free selenocysteine and is incorporated specifi cally into formate dehydrogenase H and tRNA nucleosides. J Bacteriol. 2002; 184: 1940–1946
152 Behne D, Kyriakopoulos A, Kalcklosch M, Weiss-Nowak C, Pfeifer H, Gessner H, Hammel C. Two new selenoproteins found in the prostatic glandular epithelium and in the spermatid nuclei. Biomed Environ Sci. 1997 Sep; 10(2-3): 340-5.
153 Gladyshev VN, Jeang KT, Wootton JC, Hatfi eld DL. A new human selenium-containing protein. Purification, characterization and cDNA sequence. J Biol Chem. 1998; 273: 8910–8915
154 Kumaraswamy E, Malykh A, Korotkov KV, Kozyavkin S, Hu Y, Kwon SY, Moustafa ME, Carlson BA, Berry MJ, Lee BJ, Hatfield DL, Diamond AM, Gladyshev VN. Structure-expression relationships of the 15-kDa selenoprotein gene. Possible role of the protein in cancer etiology. J Biol Chem. 2000; 275: 35540–35547
155 Apostolou S, Klein JO, Mitsuuchi Y, Shetler JN, Poulikakos PI, Jhanwar SC, Kruger WD, Testa JR. Growth inhibition and induction of apoptosis in mesothelioma cells by selenium and dependence on selenoprotein SEP15 genotype. Oncogene 2004; 23: 5032–5040
156 Korotkov KV, Kumaraswamy E, Zhou Y, Hatfield DL, Gladyshev VN. Association between the 15-kDa selenoprotein and UDP-glucose:glycoprotein glucosyltransferase in the endoplasmic reticulum of mammalian cells. J Biol Chem. 2001; 276: 15330–15336
157 Arnold SM, Fessler LI, Fessler JH, Kaufman RJ. Two homologues encoding human UDP-glucose: glycoprotein glucosyltransferase differ in mRNA expression and enzymatic activity. Biochemistry 2000; 39: 2149–2163
158 Kumaraswamy E, Korotkov KV, Diamond AM, Gladyshev VN, Hatfi eld DL. Genetic and functional analysis of mammalian Sep15 selenoprotein. Methods Enzymol. 2002; 347: 187–197
159 Kyriakopoulos A, Bertelsmann H, Graebert A, Hoppe B, Kuhbacher M, Behne D. Distribution of an 18 kDa-selenoprotein in several tissues of the rat. J Trace Elem Med Biol. 2002;16(1):57-62.
160 Kyriakopoulos A, Rothlein D, Pfeifer H, Bertelsmann H, Kappler S, Behne D. Detection of small selenium-containing proteins in tissues of the rat. J Trace Elem Med Biol. 2000 Oct;14(3):179-83
161 Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN. Characterization of mammalian selenoproteomes. Science 2003; 300: 1439–1443
162 Zhang QH, Ye M, Wu XY, Ren SX, Zhao M, Zhao CJ, Fu G, Shen Y, Fan HY, Lu G, Zhong M, Xu XR, Han ZG, Zhang JW, Tao J, Huang QH, Zhou J, Hu GX, Gu J, Chen SJ, Chen Z. Cloning and functional analysis of cDNAs with open reading frames for 300 previously undefi ned genes expressed in CD34+ hematopoietic stem/progenitor cells. Genome Res. 2000; 10: 1546–1560
163 Korotkov KV, Novoselov SV, Hatfi eld DL, Gladyshev VN. Mammalian selenoprotein in which selenocysteine (Sec) incorporation is supported by a new form of Sec insertion sequence element. Mol Cell Biol. 2002; 22: 1402–1411
164 Moghadaszadeh B, Petit N, Jaillard C, Brockington M, Roy SQ, Merlini L, Romero N, Estournet B, Desguerre I, Chaigne D, Muntoni F, Topaloglu H, Guicheney P. Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome. Nat. Genet. 2001; 29: 17–18
165 Petit N, Lescure A, Rederstorff M, Krol A, Moghadaszadeh B, Wewer UM, Guicheney P. Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern. Hum Mol Genet. 2003; 12: 1045–1053
166 Ferreiro A, Ceuterick-de Groote C, Marks JJ, Goemans N, Schreiber G, Hanefeld F, Fardeau M, Martin JJ, Goebel HH, Richard P, Guicheney P, Bonnemann CG. Desmin-related myopathy with Mallory body-like inclusions is caused by mutations of the selenoprotein N gene. Ann Neurol. 2004; 55: 676–686
167 Ferreiro A, Quijano-Roy S, Pichereau C, Moghadaszadeh B, Goemans N, Bönnemann C, Jungbluth H, Straub V, Villanova M, Leroy JP, Romero NB, Martin JJ, Muntoni F, Voit T, Estournet B, Richard P, Fardeau M, Guicheney P. Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multiminicore disease: reassessing the nosology of early-onset myopathies. Am J Hum Genet. 2002; 71: 739–749
168 Kryukov GV, Kryukov VM, Gladyshev VN. New mammalian selenocysteine-containing proteins identified with an algorithm that searches for selenocysteine insertion sequence elements. J Biol Chem. 1999; 274: 33888–33897
169 Kryukov GV, Kumar RA, Koc A, Sun Z, Gladyshev VN. Selenoprotein R is a zinc-containing stereo-specific methionine sulfoxide reductase. Proc Natl Acad Sci. USA 2002; 99: 4245–4250

170 Kim HY, Gladyshev VN. Characterization of mouse endoplasmic reticulum methionine-R-sulfoxide reductase. Biochem Biophys Res Commun. 2004; 320: 1277–1283
171 Grimaud R, Ezraty B, Mitchell JK, Lafitte D, Briand C, Derrick PJ, Barras F. Repair of oxidized proteins. Identification of a new methionine sulfoxide reductase. J Biol Chem. 2001; 276: 48915–48920
172 Boschi-Muller S, Olry A, Antoine M, Branlant G. The enzymology and biochemistry of methionine sulfoxide reductases. Biochim Biophys Acta. 2005; 1703: 231–238
173 Stadtman ER, Moskovitz J, Levine RL. Oxidation of methionine residues of proteins: biological consequences. Antioxid Redox Signal. 2003; 5: 577–582
174 Hansel A, Heinemann SH, Hoshi T. Heterogeneity and function of mammalian MSRs: enzymes for repair, protection and regulation. Biochim Biophys Acta 2005; 1703: 239–247
175 Gao Y, Walder K, Sunderland T, Kantham L, Feng HC, Quick M, Bishara N, de Silva A, Augert G, Tenne-Brown J, Collier GR. Elevation in Tanis expression alters glucose metabolism and insulin sensitivity in H4IIE cells. Diabetes 2003; 52: 929–934
176 Walder K, Kantham L, McMillan JS, Trevaskis J, Kerr L, De Silva A, Sunderland T, Godde N, Gao Y, Bishara N, Windmill K, Tenne-Brown J, Augert G, Zimmet PZ, Collier GR. Tanis: a link between type 2 diabetes and inflammation? Diabetes 2002; 51: 1859–1866
177 Gao Y, Feng HC, Walder K, Bolton K, Sunderland T, Bishara N, Quick M, Kantham L, Collier GR. Regulation of the selenoprotein SelS by glucose deprivation and endoplasmic reticulum stress– SelS is a novel glucose-regulated protein. FEBS Lett. 2004; 563: 185–190
178 Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. Nature 2004; 429: 841–847
179 Vendeland SC, Beilstein MA, Chen CL, Jensen ON, Barofsky E, Whanger PD. Purifi cation and properties of selenoprotein W from rat muscle. J Biol Chem. 1993; 268: 17103–17107
180 Yeh JY, Beilstein MA, Andrews JS, Whanger PD. Tissue distribution and influence of selenium status on levels of selenoprotein W. FASEB J. 1995; 9: 392–396
181 Yeh JY, Gu QP, Beilstein MA, Forsberg NE, Whanger PD. Selenium infl uences tissue levels of selenoprotein W in sheep. J Nutr. 1997; 127: 394–402
182 Whanger PD. Selenoprotein W. Methods Enzymol. 2002; 347: 179–187
183 Yeh JY, Ou BR, Gu QP, Whanger PD. Influence of gender on selenoprotein W, glutathione peroxidase and selenium in tissues of rats. Comp. Biochem Physiol B Biochem Mol Biol. 1998; 119: 151–155
184 Bellingham J, Gregory-Evans K, Fox MF, Gregory-Evans CY. Gene structure and tissue expression of human selenoprotein W, SEPW1, and identification of a retroprocessed pseudogene, SEPW1P. Biochim Biophys Acta 2003; 1627: 140–146
185 Beilstein MA, Vendeland SC, Barofsky E, Jensen ON, Whanger PD. Selenoprotein W of rat muscle binds glutathione and an unknown small molecular weight moiety. J Inorg Biochem. 1996; 61: 117–124
186 Bauman AT, Malencik DA, Barofsky DF, Barofsky E, Anderson SR, Whanger PD. Selective production of rat mutant selenoprotein W with and without bound glutathione. Biochem Biophys Res Commun. 2004; 313: 308–313
187 Gu QP, Beilstein MA, Barofsky E, Ream W, Whanger PD. Purifi cation, characterization, and glutathionebinding to selenoprotein W from monkey muscle. Arch Biochem Biophys. 1999; 361: 25–33
188 Sun Y, Gu QP, Whanger PD. Selenoprotein W in overexpressed and underexpressed rat glial cells in culture. J Inorg Biochem. 2001; 84: 151–156
189 Jeong D, Kim TS, Chung YW, Lee BJ, Kim IY. Selenoprotein W is a glutathione-dependent antioxidant in vivo. FEBS Lett. 2002; 517: 225–228
190 Tolman EL, Barris E, Burns M, Pansini A, Partridge R. Effects of vanadium on glucose metabolism in vitro. Life Sci. 1979; 20: 1159 –1164
191 Shechter Y, Karlish SJD. Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (IV) ions. Nature 1988; 284: 556-558
192 Dubyak GR, Kleinzeller A. Insulin-mimetic effects of vanadate in isolated rat adipocytes. J Biol Chem. 1980; 255: 5306-5312
193 Mountjoy KG, Flier JS. Vanadate regulates glucose transporter (GLUT-1) expression in NIH3T3 mouse fibroblasts. Endocrinology 1990; 126: 2778-2787
194 Tamura S, Brown TA, Whipple JH, Yamaguchi YF, Dubler RE, Cheng K. A novel mechanism for the insulin-like effect of vanadate on glycogen synthase in the rat adipocytes. J Biol Chem. 1984; 259: 6650-6656
195 Sekar N, Li J, He Z, Gefel D, Schechter Y. Independent signal-transduction pathways for vanadate and for insulin in the activation of glycogen synthesis and glycogenesis in rat adipocytes. Endocrinology 1999; 140: 1125-1131
196 Pugazhenti S, Khandeval RL. Does the insulin-mimetic action of vanadate involve insulin receptor kinase? Mol Cell Biochem 1993; 127: 211-218
197 Pederson RA, Ramanadham S, Buchan AMJ, McNeill JH. Long-term effects of vanadyl treatment on streptozotocin-induced diabetic rats. Diabetes 1989; 38: 1390-1395

198 Sakurai H, Tschuiya K, Nukatsuka M, Sofue M, Kawada J. Insulin-like effects of vanadyl ion on streptozotocin induced dibetic rats. J Endocrinol 1990; 126: 451-455
199 Brichard SM, Bailey CJ, Henquin JC. Marked improvement of glucose homeostasis in diabetic ob/ob mice given oral vanadate. Diabetes 1990; 39: 1326-1332
200 Mohammad A, Wang J, McNeill JH. Bis(maltolato)oxovanadium (IV) inhibits the activity of PTP1B in Zucker rat skeletal muscle. Mol Cell Biochem. 2002; 229: 125-128
201 Schmid AC, Byrne RD, Vilar R, Woscholski R. Bisperoxovanadium compounds are potent PTEN inhibitors. FEBS Lett. 2004; 566: 35-38
202 Ezaki O. The insulin-like effect of selenate in rat adipocytes. J Biol Chem. 1990; 262: 6658-6662
203 Furnsinn C, Englisch R, Ebner K, Nowotny P, Vogle C, Waldhausl W. Insulin-like vs. non-insulin stimulation of glucose metabolism by vanadium tungsten and selenium compounds in rat muscle. Life Sci 1996; 59: 1989-2000
204 McNeill JH, Delgatty HL, Battell ML. Insulinlike effects of sodium selenate in streptozotocin-induced diabetic rats. Diabetes 1991; 40: 1675-1678
205 Battell ML, Delgatty HL, McNeill JH. Sodium selenate corrects glucose tolerance and heart function in STZ diabetic rats. Mol Cell Biochem. 1998; 179: 27-34
206 Sheng XQ, Huang KX, Xu HB. New experimental observation on the relationship of selenium and diabetes. Biol Trace Elem Res. 2004; 99: 241-253
207 Girard J, Perdereau D, Foufelle F, Prip-Buus C, Ferre P. Regulation of lipogenic enzyme gene expression by nutrients and hormones. FASEB J. 1994; 8: 36–42
208 O'Brien RM, Streeper RS, Ayala JE, Stadelmaier BT, Hornbuckle LA. Insulin-regulated gene expression. Biochem Soc Trans. 2001; 29: 552–558
209 Bosch R, Hatzoglou M, Park EA, Hanson RW. Vanadate inhibits the expression of the gene for phoephoenolpyruvate carboxykinase. J Biol Chem. 1990; 265: 13677-13682
210 Becker DJ, Reul B, Ozcelikay AT, Buchet JP, Henquin JC, Brichard SM. Oral selenate improves glucose homeostasis and partly reverses abnormal expression of liver glycolytic and gluconeogenic enzymes in diabetic rats. Diabetologia 1996; 39: 3-11
211 Berg EA, Wu JY, Campbell L, Kagey M, Stapleton SR. Insulin-like effects of vanadate and selenate on the expression of glucose-6-phosphate dehydrogenase and fatty acid synthase in diabetic rats. Biochimie 1995; 77: 919-924
212 Stapleton SR, Jivraj S, Wagle A. Insulin and the insulin-mimetic selenium mediate the regulation of glucose-6-phosphate-dehydrogenase gene expression via different signal proteins. FASEB J. 1998; 12: 1404
213 Zhu Y, Goodridge AG, Stapleton SR. Zinc, vanadate and selenate inhibit the tri-iodothyronine-induced expression of fatty acid synthase and malic enzyme in chick-embryo hepatocytes in culture. Biochem J. 1994;303:213-216.
214 Xu P, Jacobs AR, Taylor SI. Interaction of insulin receptor substrate 3 with insulin receptor, insulin receptor-related receptor, insulin-like growth factor-1 receptor, and downstream signaling proteins. J Biol Chem. 1999; 274: 15262-70
215 Pillay TS, Makgoba MW. Enhancement of EGF and insulin-stimulated tyrosine phosphorylation of endogenous substrates by sodium selenate. FEBS Lett. 1992; 308: 38-42
216 Okada T, Kawanok Y, Sakakibara T, Hazeki O, Uli M. Essential role of phosphatidyl inositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. J Biol Chem. 1994; 269: 3568-3573
217 Stapleton SR, Garlock GL, Foellmi-Adams L, Kletzien RE. Selenium potent stimulator of tyrosyl phosphorylation and activator of MAP kinase. Biochim Biophys Acta. 1997; 1355: 259-269
218 Hei YJ, Farahbakshian S, Chen X, Battel, ML, McNeill JH. Stimulation of MAP kinase and S6 kinase by vanadium and selenium in rat adipocytes. Mol Cell Biochem. 1998; 178: 367-375
219 McClung JP, Ronecker CA, Weipeng M, Lisk DJ, Langlais P, Liu F, Lei XG. Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. Proc Natl Acad Sci. 2004; 101: 8852 – 8857
220 Lei XG, Cheng WH. New roles for an old selenoenzyme: evidence from glutathione peroxidase 1 null and overexpressing mice. J Nutr. 2005; 135: 2295 – 2298
221 Chen X, Scholl TO, Leskiw MJ, Donaldson MR, Stein TP. Association of glutathione peroxidase activity with insulin resistance and dietary fat intake during normal pregnancy. J Clin Endocrinol Metab. 2003; 88: 5963 – 5968
222 Dong F, Fang CX, Yang X, Zhang X, Lopez FL, Ren J. Cardiac overexpression of catalase rescues cardiac contractile dysfunction induced by insulin resistance: role of oxidative stress; protein carbonyl formation and insulin sensitivity. Diabetologia 2006; 49: 1421 – 1433
223 Goldstein BJ, Ahmad F, Ding W, Li PM, Zhang WR. Regulation of the insulin signalling pathway by cellular protein tyrosine phosphatases. Mol Cell Biochem. 1998; 182: 91 – 99

224 Dube N, Tremblay ML. Involvement of the small protein tyrosine phosphatases TC-PTP and PTP1B in signal transduction and diseases: From diabetes, obesity to cell cycle and cancer. Biochim Biophys Acta. 2005; 1754: 108 – 117
225 Tonks NK. PTP1B: From the sidelines to the front lines! FEBS Lett. 2003; 546: 140–148
226 Kenner KA, Anyanwu E, Olefsky JM, Kusari J. Protein tyrosine phosphatase 1B is a negative regulator of insulin- and insulin-like growth factor-I-stimulated signalling. J. Biol Chem. 1996; 271: 19810 – 19816
227 Joost HG, Giesen K, Kluge R, Ortlepp J, Plum L. Insulin resistance and metabolic syndrome. Z Kardiol. 2000; 89: 377 – 382
228 Doelle GC. The clinical picture of metabolic syndrome. An update on this complex of conditions and risk factors. Postgrad Med. 2004; 116: 30 – 32 and 35-38.
229 Laaksonen DE, Niskanen L, Lakka HM, Lakka TA, Uusitupa M. Epidemiology and treatment of the metabolic syndrome. Ann Med. 2004; 36: 332-346
230 Ahmad F, Considine RV, Bauer TL, Ohannesian JP, Marco CC, Goldstein BJ. Improved sensitivity to insulin in obese subjects following weight loss is accompanied by reduced protein tyrosine phosphatases in adipose tissue. Metabolism 1997; 46: 1140 – 1145
231 Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 1999; 283: 1544–1548
232 Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Scarpe AH, Stricker-Krongard A, Shulman GI, Neel BG, Kahn BB. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol. 2000; 20: 5479–89
233 Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, Xie N, Wilcox D, Jacobson P, Frost I, Kroeger PE, Reilly RM, Koterski S, Opgenorth TJ, Ulrich RG, Crosby S, Butler M, Murray SF, McKay R, Bhanot S, Monia BP, Jirousek MR. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci. 2002; 99: 11357 – 11362
234 Gum RJ, Gaede, LL, Koterski SL, Heindel M, Clampit JE, Zinker BA, Trevilyan JM, Ulrich RG, Jirousek MR, Rondinone CM. Reduction of protein tyrosine phosphatase 1B increases insulin-dependent signaling in ob/ob mice. Diabetes 2003; 52: 21 – 28
235 Ramachandran C, Kennedy BP. Protein tyrosine phosphatase 1B: a novel target for type 2 diabetes and obesity. Curr Top Med Chem. 2003; 3: 749 – 757
236 Huyer G, Liu S, Kelly J, Moffat J, Payette P, Kennedy B, Tsaprailis G, Gresser MJ, Ramachandran C. Mechanism of inhibition of protein tyrosine phosphatases by vanadate and pervanadate. J Biol Chem. 1997; 272; 843 - 851
237 Denu JM, Dixon JE. Protein tyrosine phosphatases: mechanisms of catalysis and regulation. Curr Opin Chem Biol. 1998; 2: 633 - 641
238 Denu JM, Tanner KG. Redox regulation of protein tyrosine phosphatases by hydrogen peroxide: detecting sulphenic acid intermediates and examining reversible inactivation. Methods Enzymol. 2002; 348: 297 - 305
239 Salmeen A, Andersen JN, Myers MP, Meng TZ, Hinks,JA, Tonks NK, Barford D. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. Nature 2003; 423: 769-773
240 Van Montfort RL, Congreve M, Tisi D, Carr R, Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. Nature 2003; 423: 773-777
241 Barrett WC, DeGnore JP, Koenig S, Fales HM, Keng YF, Zhang ZY, Yim MB, Chock PB. Regulation of PTP1B via glutathionylation of the active site cysteine 215. Biochemistry 1999; 28: 6699 – 6705
242 Mahadev K, Zilbering A, Zhu L, Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1B in vivo and enhances the early insulin action cascade. J Biol Chem. 2001; 276: 21938-21942
243 Wu X, Zhu L, Zilbering A, Mahadev K, Motoshima H, Yao J, Goldstein BJ. Hyperglycemia potentiates H_2O_2 production in adipocytes and enhances insulin signal transduction: potential role for oxidative inhibition of thiol-sensitive protein tyrosine phosphatases. Antioxid Redox Signal. 2005; 7: 526 – 537
244 Johnson LW, Weinstock RS. The metabolic syndrome: concepts and controversy. Mayo Clin Proc. 2006; 81: 1615–1620
245 Lyons GH, Judson GJ, Ortiz-Monasterio I, Genc Y, Stangoulis JC, Graham RD. Selenium in Australia: selenium status and biofortification of wheat for better health. J Trace Elem Med Biol. 2005; 19: 75-82
246 Broadley MR, White PJ, Bryson RJ, Meacham MC, Bowen HC, Johhnson SE, Hawkesford MJ, McGrath SP, Zhao FJ, Breward N, Harriman M, Tucker M. Biofortification of UK food crops with selenium. Proc Nutr Soc. 2006; 65: 169-181
247 Arnault I, Auger J. Selenocompounds in garlic and onion. J Chromatogr A. 2006; 21: 23-30

248 Lintschinger J, Fuchs N, Moser J, Kuehnelt D, Goessler W. Selenium-enriched sprouts. A raw material for fortified cereal-based diets. J Agric Food Chem. 2000; 48: 5362-5368
249 Juniper DT, Philipps RH, Jones AK, Bertin G. Selenium supplementation of lactating dairy cows: effect on selenium concentration in blood, milk, urine, and faeces. J Dairy Sci. 2006; 89: 3544-3551
250 Yoon I, McMillan E. Comparative effects of organic and inorganic selenium on selenium transfer from sows to nursing pigs. J Anim Sci. 2006; 84: 1729-1733
251 Gulbas SY, Saldamli I. The effect of selenium and zinc fortification on the quality of Turkish white cheese. Int J Food Sci Nutr. 2005; 56: 141-146
252 Pappas AC, Acamovic T, Sparks NH, Surai PF, McDevitt RM. Effects of supplementing broiler breeder diets with organoselenium compounds and polyunsaturated fatty acids on egg quality during storage. Poult Sci. 2005; 84: 865 – 874
253 Greenwald P, Anderson D, Nelson SA, Taylor PR. Clinical trials of vitamin and mineral supplements for cancer prevention. Am J Clin Nutr. 2007 Jan; 85: 314S-317S
254 Mueller AS, Pallauf J, Rafael J. The chemical form of selenium affects insulinomimetic properties of the trace element: Investigations in type II diabetic dbdb mice. J Nutr Biochem. 2003; 14, 637 – 647
255 Mueller AS, Erika M, Pallauf J. Effects of a supranutritional dose of selenate compared to selenite and selenium deficiency on insulin sensitivity in type II diabetic dbdb mice. J Anim Physiol Anim Nutr. 2005; 89, 94 – 104
256 Mueller AS, Pallauf J. Compendium of the antidiabetic effects of supranutritional selenate doses. In vivo and in vitro investigations with type II diabetic dbdb mice. J Nutr Biochem. 2006; 17 (8), 548 - 560
257 Mueller AS, Bosse AC, Pallauf J. Selenium an ambivalent factor in diabetes? Established facts, Recent findings and Perspectives Current. Nutrition and Food Science. 2006; 2 (2), 151 – 168
258 Mueller AS, Bosse AC, Most E, Klomann SD, Schneider S, Pallauf J. Selenium a risk factor for diabetes development ? The regulation of PTP1B may be one part of the puzzle (submitted to J Nutr Biochem) 2007;
259 Prochazka M, Leiter EH. Effect of androgen insensitivity on diabetogenesis in db/db male mice with testicular feminization (Tfm). Horm Metab Res. 1991; 23: 149-154
260 Kitahara J, Seko Y, Imura N. Possible involvement of active oxygen species in selenite toxicity in isolated rat hepatocytes. Arch Toxicol. 1993; 67: 497-501
261 Kim EH, Sohn S, Kwon HJ, Kim SU, Kim MJ, Lee SJ, Choi KS. Sodium selenite induces superoxide-mediated mitochondrial damage and subsequent autophagic cell death in malignant glioma cells. Cancer Res. 2007; 67: 6314-6324
262 Rinna A, Torres M, Forman HJ. Stimulation of the alveolar macrophage respiratory burst by ADP causes selective glutathionylation of protein tyrosine phosphatase 1B. Free Radic Biol Med. 2006; 41: 86 – 91
263 von Montfort C, Sharov VS, Metzger S, Schoneich C, Sies H, Klotz LO. Singlet oxygen inactivates protein tyrosine phosphatase 1B by oxidation of the active site cysteine. Biol Chem. 2006; 387: 1399 – 1404
264 Bleys J, Navas-Acien A, Guallar E. Serum selenium and diabetes in U.S. adults. Diabetes Care. 2007 Apr; 30: 829-834
265 Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, Cappuccio FP, Ceriello A, Reid ME. Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. Ann Intern Med. 2007 Aug 21; 147: 217-223
266 Jung UJ, Lee MK, Park YB, Kang MA, Choi MS. Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. Int J Biochem Cell Biol. 2006; 38: 1134-1145
267 Hori M, Satoh M, Furukawa K, Sakamoto Y, Hakamata H, Komohara Y, Takeya M, Sasaki Y, Miyazaki A, Horiuchi S. Acyl-coenzyme A: cholesterol acyltransferase-2 (ACAT-2) is responsible for elevated intestinal ACAT activity in diabetic rats. Arterioscler Thromb Vasc Biol. 2004; 24; 1689 – 1695
268 Suzuki D, Toyoda M, Yamamoto N, Miyauchi M, Katoh M, Kimura M, Maruyama M, Honma M, Umezono T, Yagame M. Relationship between the expression of advanced glycation end products (AGE) and the receptor for AGE (RAGE) mRNA in diabetic nephropathy. Intern Med. 2006; 45: 435 - 441
269 Yamagishi S, Imaizumi T. Serum levels of soluble form of receptor for advanced glycation end products (sRAGE) may reflect tissue RAGE expression in diabetes. Arterioscler Thromb Vasc Biol. 2007; 27: E 32
270 Li G, Zhang Y, Cheng KY, Scarpace PJ. Lean rats with hypothalamic pro-opiomelanocortin overexpression exhibit greater diet-induced obesity and impaired central melanocortin responsiveness. Diabetologia 2007; 50: 1490-1499.
271 Gao J, Katagiri H, Ishigaki Y, Yamada T, Ogihara T, Imai J, Uno K, Hasegawa Y, Kanzaki M, Yamamoto TT, Ishibashi S, Oka Y. Involvement of apolipoprotein E in excess fat accumulation and insulin resistance. Diabetes 2007; 56: 24 - 33

272 Alborn WE, Prince MJ, Konrad RJ. Relationship of apolipoprotein A 5 and apolipoprotein C3 levels to serum triglycerides in patients with type 2 diabetes. Clin Chim Acta. 2007; 378: 154 – 158
273 Li Z, Murakami T, Nakai N, Nagasaki M, Obayashi M, Xu M, Sato J, Oshida Y, Sato Y, Shimomura Y. Modification by exercise training of activity and enzyme expression of hepatic branched-chain alpha-ketoacid dehydrogenase complex in streptozotocin-induced diabetic rats. J Nutr Sci Vitaminol. (Tokyo) 2001; 47: 345-350
274 Harris RA, Powell SM, Paxton R, Gillim SE, Nagae H. Physiological covalent regulation of rat liver branched-chain alpha-ketoacid dehydrogenase. Arch Biochem Biophys. 1985; 43: 542-555
275 Nakada N, Kuroda K, Kawahara E. Protein phosphatase 2A regulatory subunit Bbeta promotes MAP kinase-mediated migration of A431 cells. Cell Physiol Biochem. 2005; 15: 19 – 28
276 Wu Y, Song P, Xu J, Zhang M, Zou MH. Activation of protein phosphatase 2A by palmitate inhibits AMP-activated protein kinase. J Biol Chem. 2007; 282: 9777: 9788
277 Ravnskjaer K, Boergesen M, Dalgaard LT, Mandrup S. Glucose-induced repression of PPARalpha gene expression in pancreatic beta cells involves PPA2A activation and AMPK inactivation. J Mol Endocrinol. 2006; 36: 289 – 299
278 AMP-activated protein kinase inhibits the glucose-activated expression of fatty acid synthase gene in rat hepatocytes. J Biol Chem. 1998; 273: 14767 – 14771
279 Shimizu S, Ugi S, Maegawa H, Egawa K, Nishio Y, Yoshizaki T, Shi K, Nagai Y, Morino K, Nemoto K, Nakamura T, Bryer-Ash M, Kashiwagi A. Protein tyrosine phosphatase 1B as new activator for hepatic lipogenesis via sterol regulatory element-binding protein 1 gene expression. J Biol Chem. 2003; 278: 43095 – 43101
280 Richard CL, Blay J. Thiazolidinedione drugs down-regulate CXCR4 expression on human colorectal cancer cells in a peroxisome proliferator activated receptor gamma-dependent manner. Int J Oncol. 2007; 30: 1215 – 1222
281 Lieber CS, Leo MA, Mak KM, Xu Y, Cao Q, Ren C, Ponomarenko A, DeCarli LM. Model of nonalcoholic steatohepatitis. Am J Clin Nutr. 2004;79: 502-509
282 Chan O, Inouye K, Akirav E, Park E, Riddell MC, Vranic M, Matthews SG. Insulin alone increases hypothalamo-pituitary-adrenal activity, and diabetes lowers peak stress responses. Endocrinology 2005; 146: 1382-1390.
283 Doyon C, Samson P, Lalonde J, Richard D. Effects of the CRF1 receptor antagonist SSR125543 on energy balance and food deprivation-induced neuronal activation in obese Zucker rats. J Endocrinol. 2007; 193: 11-19
284 C-reactive protein and features of metabolic syndrome in Brazilian women with previous gestational diabetes. Diabetes Res Clin Pract. 2007, April, epub ahead of print
285 Nystrom T. C-reaktive protein: a marker or a player? Clin Sci. 2007, April, epub ahead of print
286 Muda M, Boschert U, Dickinson R, Martinou JC, Martinou I, Camps M, Schlegel W, Arkinstall S. MKP-3, a novel cytosolic protein-tyrosine phosphatase that exemplifies a new class of mitogen-activated protein kinase phosphatase. J Biol Chem. 1996; 271: 4319 - 4326
287 Newberry EP, Xie Y, Kennedy SM, Luo J, Davidson NO. Protection against Western diet-induced obesity and hepatic steatosis in liver fatty acid-binding protein knockout mice. Hepatology 2006; 44: 1191-205
288 van Poelje PD, Potter SC, Chandramouli VC, Landau BR, Dang Q, Erion MD. Inhibition of fructose 1,6-bisphosphatase reduces excessive endogenous glucose production and attenuates hyperglycemia in Zucker diabetic fatty rats. Diabetes 2006; 55: 1747-1754
289 Wu C, Okar DA, Newgard CB, Lange AJ. Increasing fructose-2,6-bisphosphate overcomes hepatic insulin resistance of type 2 diabetes. Am J Physiol Endocrinol Metab. 2002; 282: E38 -
290 Wang D, Wei Y, Schmoll D, Maclean KN, Pagliasotti MJ. Endoplasmatic reticulum stress increases glucose-6-phosphatase and glucose cycling in liver cells. Endocrinology 2006; 147: 350 – 358
291 Abdollahi M, Donyavi M, Pournourmohammadi S, Saadat M. Hyperglycemia associated with increased hepatic glycogen phosphorylase and and phosphoenolpyruvate carboxykinase in rats following subchronic exposure to malathion. Comp Biochem Physiol C Toxicol Pharmacol. 2004; 137: 343 – 347
292 Liu H, McNeill JH. Effects of vanadium treatment on the alterations of cardiac glycogen phosphorylase and phosphorylase kinase in streptozotocin-induced chronic diabetic rats. Can J Physiol Pharmacol. 1994; 72: 1537 – 1543
293 Adreeva IE, Rice NA, Carlson GM. The regulatory alpha subunit of phsophorylase kinase may directly partecipate in the binding of glycogen phosphorylase. Biochemistry 2002; 67: 1197 - 1202

294 Hampson LJ, Mackin P, Agius L. Stimulation of glycogen synthesis and inactivation of phosphorylase in hepatocytes by srotonergic mechanisms, and counter-regulation by atypical antipsychotic drugs. Diabetologia 2007; Jun 20: Epub ahead of print
295 Li M, Peterson S, Husney D, Inaba M, Guo K, Kappas A, Ikehara S, Abraham NG. Long-lasting expression of HO-1 delays progression of type I diabetes in NOD mice. Cell Cycle. 2007; 6: 567 – 571
296 Hu CM, Lin HH Chiang MT, Chang PF, Chau LY. Systemic expression of heme oxygenase-1 ameliorates type 1 diabetes in NOD mice. Diabetes 2007; 56: 1240 – 1247
297 Li Yx, Li G, Dong WP, Lu DR, Tan JM. Protection of human islets from induction of apoptosis and improved islet function with HO-1 gene transduction. Chin Med J. 2006; 119: 1639 – 1645
298 Furudono Y, Ando C, Yamamoto C, Kobashi M, Yamamoto T. Involvement of specific orexigenic neuropeptides in sweetener-induced overconsumption in rats. Behav Brain Res. 2006; 175: 241 – 248
299 Branowska B, Wolinska-Witort E, Martynska L, Chmielowska M, Baranowska-Bik A. Plasma orexin, orexin B, leptin, neuropeptide Y (NPY) and insulin in obese women. Neuro Endocrinol Lett. 2005; 26: 293 – 296
300 Park ES, Seong JK, Yi SJ, Kim JS, Lee IS, Yoon YS. Changes in orexin-A and neuropeptide Y expression in the hypothalamus of obese and lean Zucker diabetic fatty rats. J Vet Med Sci. 2005; 67: 639 – 646
301 Heionen MV, Purhonen AK, Miettinen P, Paakkonen M, Pirinen E, Alhava E, Akermann K, Herzig KH. Apelin, orexin-A, and leptin plasma lvels in morbid obesity and effect of gastric banding. Regul Pept. 2005; 130: 7 - 13
302 Haynes AC, Jackson B, Chapman H, Tadayyon M, Johns A, Porter RA, Arch JR. A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. Regul Pept. 2000; 96: 45 – 51
303 Oshima H, Taketo MM, Oshima M. Destruction of pancreatic beta-cells by transgenic induction of prostaglandin E2 in the islets. J Biol Chem. 2006; 281: 29330-29336
304 Maddux BA, Chan A, De Filippis EA, Mandarino LJ, Goldfine ID. IGF-binding protein-1 levels are related to insulin-mediated glucose disposal and are a potential serum marker of insulin resistance. Diabetes Care. 2006; 29: 1535 – 1537
305 Bienvenu G, Seurin D, Le Bouc Y, Even P, Babajko S, Magnan C. Dysregulation of energy homeostasis in mice overexpressing insulin-like growth factor-binding protein 6 in the brain. Diabetologia 2005; 48: 1189 – 1197
306 Waite KJ, Floyd ZE, Arbour-Reily P, Stephens JM. Interferon-gamma-induced regulation of peroxisome proliferator activated receptor gamma and STATs in adipocytes. J Biol Chem. 2001; 276: 7062 – 7068
307 Jager J, Gremeaux T, Cormont M, Le-Marchand-Brustel Y, Tanti JF. Interleukin-1 beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor subtrate-1 expression. Endocrinology 2007; 148: 241 – 251
308 Choi KM, Ryu OH, Lee KW, Kim HY, Seo JA, Kim SG, Kim NH, Choi DS, Baik SH. Serum adiponectin, interleukin-10 levels and inflammatory markers in the metabolic syndrome. Diabetes Res Clin Pract. 2007; 75: 235 – 240
309 Den Boer MA, Voshol PJ, Schroder-van der Elst JP, Korshennikova E, Ouwens DM, Kuipers F, Hveskes LM, Romijn JA. Endogenous interleukin-10 protects against hepatic steatosis but does not improve insulin sensitivity during high fat feeding in mice. Endocrinology 2006; 147: 4553 - 4558
310 Phillips JM, Parish NM, Drage M, Cooke A. Cutting edge: interactions through the IL-10 receptor regulate autoimmune diabetes. J Immunol. 2001; 167: 6087 – 6091
311 Rajagopalan G, Kudva YC, Sen MM, Marietta EV, Murali N, Nath K, Moore J, David CS. IL-10 deficiency unmasks unique immune system defects and reveals differential regulation of organ-specific autoimmunity in non-obese diabetic mice. Cytokine 2006; 34, 85 – 95
312 Trabattoni D, Schenal M, Cesari M, Castelli E, Pacei M, Goldberg B, Gori A, Clerici M. Low interleukin-10 production is associated with diabetes in HIV-infected patients undergoing antiviral therapy. Med Microbiol Immunol. 2006; 195: 125 – 132
313 Cetkovic-Cvrije M, Dragt AL, Vassilev A, Liu XP, Uckun FM. Targeting JAK3 with JANEX-1 for prevention of autoimmune type 1 diabetes in NOD mice. Clin Immunol. 2003; 106:213 - 225
314 Awazu M, Ishikura K, Hida M, Hoshiya M. Mechanisms of mitogen-activated protein kinase activation in experimental diabetes. J Am Soc Nephrol. 1999; 10: 738 – 745
315 Yadav UC, Moothy K, Baguer NZ. Effects of sodium-othovanadate and Trigonella foenum-graecum seeds on hepatic and renal lipogenic enzymes and lipid profile during alloxan diabetes. J Biosci. 2004; 29: 81 – 91
316 Khandelwal RL, Pugazhenti S. In vivo effects of vanadate on hepatic glycogen metabolizing and lipogenic enzymes in insulin-dependent and insulin-resistant diabetic animals. Mol Cell Biochem. 1995; 153: 87 – 94

317 Wu JJ, Roth RJ, Anderson EJ, Hong EG, Lee MK, Choi CS, Neufer PD, Shulman GI, Kim JK, Bennett AM. Mice lacking MAP kinase phosphatase-1 have enhanced MAP kinase activity and resistance to diet-induced obesity. Cell Metab. 2006; 4: 61 – 73
318 Xu H, Dembski, M, Yang Q, Yang D, Moriarty A, Tayber O, Chen H, Kapeller R, Tartaglia LA. Dual specificity mitogen-activated protein (MAP) kinase phosphatase-4 plays a potential role in insulin resistance. J Biol Chem. 2003; 278: 30187 – 30192
319 Xu H, Yang Q, Shen M, Huang X, Dembski M, Gimeno R, Tartaglia LA, Kapeller R, Wu Z. Dual specifity MAPK phosphatase 3 activates PEPCK gene transcription and increases gluconeogenesisin rat hepatoma cells. J Biol Chem. 2005; 280: 36013 – 36018
320 Awazu M, Ishikura K, Hida M, Hoshiya M. Mechanisms of mitogen-activated protein kinase activation in experimental diabetes. J Am Soc Nephrol. 1999; 10: 738-745.
321 Lazo JS, Nunes R, Skoko JJ, Querioz de Oliveira PE, Vogt A, Wipf P. Novel benzofuran inhibitors of human mitogen-activated protein kinase phosphatase-1. Bioorg Med Chem. 2006; 14: 5643 – 5650
322 Zhang W, Khan A, Ostenson CG, Berggren PO, Efendic S, Meister B. Down-regulated expression of exocytotic proteins in panceratic islets of GK rats. Biochem Biophys Res Commun. 2002; 291: 1038 – 1044
323 Ostenson CG, Gaisano H, Sheu L, Tibell A, Bartfai T. Impaired gene and protein expression of exocytotic soluble N-ethylmaleimide attachment protein receptor complex proteins in pancreatic islets of type 2 diabetic patients. Diabetes 2006; 55: 435-440
324 Kuo LE, Abe K, Zukowska Z. Stress, NPY and vascular remodelling for stress-related diseases. Peptides 2007; 28: 435 – 440
325 Park S, Peng XD, Frohman LA, Kineman RD. Expression analysis of hypothalamic and pituitary components of the growth hormone axis in fasted and streptozotocin-treated neuropeptide Y (NPY)-intact (NPY+/+) and NPY-knockout (NPY-/-) mice. Neuroendocrinology 2005; 81: 360-371
326 Cayón A, Crespo J, Mayorga M, Guerra A, Pons-Romero F. Increased expression of Ob-Rb and its relationship with the overexpression of TGF-beta1 and the stage of fibrosis in patients with nonalcoholic steatohepatitis. Liver Int. 2006; 26: 1065-1071
327 Laybutt DR, Preston AM, Akerfeldt MC, Kench JG, Busch AK, Biankin AV, Biden TJ. Endoplasmatic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. Diabetologia 2007; 50: 752 – 763
328 Hansotia T, Maida A, Flock G, Yamada Y, Tsukiyama K, Seino Y, Drucker DJ. Extrapancreatic incretin receptors modulate glucose homeostasis, body weight, and energy expenditure. J Clin Invest. 2007 Jan; 117(1): 143-152
329 Jin XL, Sun WX, Shi, YP, Li JH, Chen HM, Pan J. Study on differentially expressed gene of the liver of treble fatty metabolism genes mutant mice using cDNA microarray. Zhoghua Yi Xue Yi Chuan Xue Za Zhi. 2005; 22 – 27 – 30
330 Duan RD, Erlanson-Albertsson C. The effect of pretranslational regulation on synthesis of pancreatic colipase in streptozotocin-induced diabetes in rats. Pancreas 1992; 7: 465 – 471
331 Patel R, Atherton P, Wackerhage H, Singh J. Signaling proteins associated with diabetic-induced exocrine pancreatic insufficiency in rats. Ann N Y Acad Sci. 2006; 1084: 490-502
332 Burgess SC, He T, Yan Z, Lindner J, Sherry AD, Malloy CR, Browning JD, Magnuson MA. Cytosolic phosphoenolpyruvate carboxykinase does not solely control the rate of hepatic gluconeogenesis in the intact mouse liver. Cell Metab. 2007; 5: 313 – 320
333 Quinn PG, Yeagley D. Insulin regulation of PEPCK gene expression: a model for rapid and reversible modulation. Curr Drug Targets Immune Endor Metabol Disord. 2005; 5: 423 – 437
334 Sun Y, Liu S, Ferguson S, Wang L, Klepcyk P, Yun JS, Friedman JE. Phosphoenolpyruvate carboxykinase overexpression selectively attenuates insulin signalling and hepatic insulin sensitivity in transgenic mice. J Biol Chem. 2002; 277: 23301 – 23307
335 Nakagawa Y, Aoki N, Aoyama K, Shimizu H, Shimano H, Yamada N, Miyazaki H. Receptor-type protein tyrosine phosphatase epsilon is a negative regulator of insulin signalling in primary hepatocytes and liver. Zoolog Sci. 2005; 22: 169 – 172
336 Lam NT, Covey SD, Lewis TT, Oosman S, Webber T, Hsu EC, Cheung, Kieffer TJ. Leptin resistance following overexpression of protein tyrosine phosphatase 1B in liver. J Mol Endocrinol. 2006; 36: 163 – 174
337 Rondinone CM, Trevillyan JM, Clampit J, Gum RJ, Berg C, Kroeger P, Frost L, Zinker BA, Reilly R, Ulrich R, Butler M, Monia BP, Jirousek MR, Waring JF. Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. Diabetes 2002; 51: 2405 – 2411
338 Jitrapakdee S, Vidal-Puig A, Wallace JC. Anaplerotic roles of pyruvate carboxylase in mammalian tissues. Cell Mol Life Sci. 2006; 63: 843-854.

339 Becker W, Kluge R, Kantner T, Linnartz K, Korn M, Tschank G, Plum L, Giesen K, Joost HG. Differential hepatic gene expression in a polygenic mouse model with insulin resistance and hyperglycemia: evidence for a combined transcriptional dysregulation of gluconeogenesis and fatty acid synthesis. J Mol Endocrinol. 2004; 32: 195-208
340 Lechuga-Sancho AM, Arroba AI, Frago LM, Garcia-Caceres C, deCelix AD, Argente J, Chowen JA. Reduction in the number of astrocytes and their protections is associated with increased synaptic protein density in the hypothalamus of poorly controlled diabetic rats. Endocrinology 2006; 147: 5314 – 5324
341 Smoak IW. Hyperglycemia induced TGFbeta and fibronectin expression in embryonic mouse heart. Dev Dyn. 2004; 231: 179 – 189
342 Lane PH, Snelling DM, Langer WJ. Streptozotocin diabetes elevates all isoforms of TGF-beta in the rat kidney. Int J Exp Diabetes Res. 2001; 2: 55 – 62
343 Schinner S, Scherbaum WA, Borsnstein SR, Barthel A. Molecular mechanisms of insulin resistance. Diab Med. 2005; 22: 674 – 682
344 Puigserver P, Rhee J, Denovan J, Walkey CJ, Yoon JC, Oriente F, Kitamura Y, Altomonte J, Dong H, Accili D, Spiegelman BM. Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. Nature 2003; 423: 550 – 555
345 Yvan-Charvet L, Even P, Bloch-Faure M, Guerre-Millo M, Moustaid-Moussa N, Ferre P, Quigard-Boulange A. Depletion of the angiotensin type 2 receptor (AT2R) reduces adipose cell size and protects from diet-induced obesity and insulin resistance. Diabetes 2005; 54: 991 – 999
346 Silha JV, Krsek M, Sucharda P, Murphy LJ. Angiogenic factors are elevated in overweight and obese individuals. Int J Obes. 2005; 29: 1308 – 1314

347 Cao Y. Angiogenesis modulates adipogenesis and obesity. J Clin Invest. 2007; 117: 2362-2368
348 Miyazawa-Hoshimoto S, Takahashi K, Bujo H, Hashimoto N, Saito Y. Elevated serum vascular endothelial growth factor is associated with visceral fat accumulation in human obese subjects. Diabetologia 2003; 46: 1483-1488
349 Sunde RA. What can molecular biology tell us about selenium requirements? In: Zimmermann, NG (ed): Proc 3nd Mid-Atlantic Nutrition Conference 2005, University of Maryland, College Park, MD 20742: 8–16
350 Cabe PA, Carmichael NG, Tilson HA. Effects of selenium, alone and in combination with silver or arsenic, in rats. Neurobehav Toxicol. 1979; 1: 275-278
351 Schaefer K, Kyriakopoulos A, Gessner H, Grune T. Effects of selenium deficiency on fatty acid metabolism in rats fed fish oil-enriched diets. J Trace Elem Med Biol. 2004; 18: 89–97
352 Wu Y, Zu K, Warren MA, Wallace PK, Ip C. Delineating the mechanism by which selenium deactivates Akt in prostate cancer cells. Mol Cancer Therap. 2006; 5: 246 – 252
353 Funchal C, Moretto MB, Vivian L, Zeni G, Rocha JBT, Pessoa-Pureur R. Diphenyl ditelluride- and methylmercury-induced hyperphosphorylation of the high molecular weight neurofilament subunit is prevented by organoselenium compounds in cerebral cortex of young rats. Toxicology 2006; 222: 143 – 153
354 McMaster D, Bell N, Anderson P, Love AHG: Automated measurement of two indicators of human of human selenium status, and applicability to population studies. Clin Chem 36: 211 – 216, 1990
355 Thomson CD, Rea HM, Doesburg VM, Robinson MF: Selenium concentrations and glutathione peroxidase activities in whole blood of New Zealand residents. Br J Nutr 37: 457 – 460, 1977
356 Zachara BA, Gromadzińska J, Wąsowicz W, Zbróg Z: Red blood cell and plasma glutathione peroxidase activities and selenium concentration in patients with chronic kidney disease: A review. Acta Biochim Pol 53: 663 – 677, 2006
357 No authors listed. Summaries for patients. Long-term use of selenium supplements and risk for type 2 diabetes. Ann Intern Med. 2007; 147: I14
358 Bleys J, Navas-Acien A, Guallar E. Selenium and diabetes: more bad news for supplements. Ann Intern Med. 2007; 147: 271-272
359 No authors listed. Selenium supplements may increase risk of type 2 diabetes. British Med J. 2007; 335: 473
360 Mueller AS, Bosse AC and Pallauf J. Selenium and diseases: Cancer or Diabetes? A few micrograms make a difference! Rapid Response to Annals of Internal Medicine: http://www.annals.org/cgi/eletters/147/4/217#23988
361 Hernandez-Montes E, Pollard SE, Vauzour D, Jofre-Montseny L, Rota C, Rimbach G, Weinberg PD, Spencer JP. Activation of glutathione peroxidase via Nrf1 mediates genistein´s protection against oxidative endothelial cell injury. Biochem Biophys Res Commun. 2006; 346: 851 – 859
362 Lee JS. Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats. Life Sci. 2006; 79: 1578 – 1584
363 Campbell L, Howie F, Arthur JR, Nicol F, Becket G. Selenium and sulforaphane modify the expression of selenoenzymes in the human endothelial cell line EAhy 926 and protect cells from oxidative damage. Nutrition 2007; 23: 138 – 144

9. Attachment 1

9.1 Declarations (Erklärungen)

9.1.1 Eigenständigkeitserklärung und Erklärung zur Einhaltung der Grundsätze guter wissenschaftlicher Praxis

Ich erkläre: „Ich habe die Habilitationsschrift „Specific physiological features of inorganic selenium compounds regarding metabolism - in vivo and in vitro investigations with type II diabetic dbdb mice and healthy rats" selbständig und nur mit den Hilfen angefertigt, die ich in der Arbeit angegeben habe.
Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.
Bei der Erstellung der Habilitationsschrift und bei den von mir durchgeführten und in der Arbeit erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität zur Sicherung guter wissenschaftlicher Praxis" umschrieben sind, eingehalten."

Giessen, 10.11.2007 Dr. Andreas Müller

9.1.2 Erklärung bezüglich der Fachgebiete der Habilitation

Mit dieser Erklärung möchte ich darlegen, dass ich die Habilitation für die Fachgebiete

„Ernährungsphysiologie“

und

„Tierernährung“

anstrebe.

Giessen, 10.11.2007 Dr. Andreas Müller

9.2 Thanks (Danksagung)

Ein kurzer Dank zum Schluss der Habilitationsschrift ist gerichtet an:

- Herrn Prof. Dr. Josef Pallauf für die Möglichkeit am Institut für Tierernährung und Ernährungsphysiologie die vorliegende Habilitationsschrift anzufertigen sowie für seine stets gewährte Hilfsbereitschaft in allen Bereichen bei der Bearbeitung des Themas.
- Herrn Prof. Dr. Johannes Rafael (Heidelberg), bei dem ich ein Jahr meiner Postdoktorandenzeit verbringen durfte, für seine Unterstützung bei den in Heidelberg begonnenen Versuchen.
- Frau Dr. Erika Most, die sich gerade in den vergangenen Jahren, die von der Neubesetzung der Professuren am Institut für Tierernährung und Ernährungsphysiologie geprägt waren, sehr für die Belange des Instituts eingesetzt hat und mich in der Grundlagenanalytik für die Versuche unterstützt hat.
- Die technischen Assistenten des Instituts für ihre Hilfe bei den Laborarbeiten und den Tierversuchen, im Einzelnen: Marco Jäger, †Steffen Brückel, Helmut Henzel, Herbert Kirch, Christian Spangenberg, Anika Fischer, Anja Marx, Susanne Breitstadt, Frauke Frank, Tina Schneider, Silke Hees und Andreas Breitstadt (ich hoffe ich habe niemanden vergessen).
- Meine Diplom,-Bachelor- und Masterstudenten, die mich im Rahmen ihrer Examensarbeiten bei den Laboranalysen unterstützt haben.
- Mein Bürogegenüber Herrn Dipl. oec. troph Klaus Brandl für echt viel Spaß und Aufmunterung im Büro und für das Ertragen von Rauchwolken, die ihm manchmal entgegenschlugen (bis zum 01.10.2007 durfte man in öffentlichen Gebäuden in Hessen noch rauchen).

Bedanken möchte ich mich auch bei meiner lieben Frau Daniela und bei meinem kleinen Sohn Richard, die es immer wieder geschafft haben, mich durch „mehr oder weniger qualifiziertes Geschrei“ aufzumuntern.
Bei meinen Eltern und Schwiegereltern für nette Stunden im Kreis der Familie.

Im Andenken an meine geliebte Schwester Ulrike, an die ich während der Erstellung dieser Arbeit häufig denken musste.

Giessen, 10.11.2007 Dr. Andreas Müller

10. Attachment 2

Original publications of this work

ELSEVIER

Journal of Nutritional Biochemistry 14 (2003) 637–647

THE JOURNAL OF Nutritional Biochemistry

The chemical form of selenium affects insulinomimetic properties of the trace element: investigations in type II diabetic dbdb mice

Andreas S. Mueller[a,b,*], Josef Pallauf[b], Johannes Rafael[a]

[a]*Biochemie Zentrum Heidelberg, Im Neuenheimer Feld 328, D-69120, Heidelberg, Germany*
[b]*Institute of Animal Nutrition and Nutrition Physiology, Heinrich-Buff-Ring 26 – 32, D-35392, Giessen, Germany*

Received 25 October 2002; received in revised form 7 August 2003; accepted 12 August 2003

Abstract

The objective of the present study was to investigate the effects of oral selenate application in comparison to selenium deficiency and selenite treatment on the development of the diabetic status (glucose tolerance, insulin resistance and activities of glycolytic and gluconeogenic marker enzymes) in dbdb mice, representing a type II diabetic animal model. Therefore 21 adult male dbdb mice were assigned to 3 experimental groups of 7 animals each and put on a selenium deficient diet (< 0.03 mg/kg diet) based on torula yeast. Group 0Se was kept on selenium deficiency for 10 weeks while the mice of the groups SeIV and SeVI were supplemented daily with 15% of their individual LD_{50} of sodium selenite or sodium selenate in addition to the diet. After 10 weeks a distinct melioration of the diabetic status indicated by a corrected glucose tolerance and a lowered insulin resistance was measured in selenate treated mice (group SeVI) in comparison to their selenium deficient and selenite treated companions and to their initial status. Activities of the glycolytic marker enzymes hexokinase, phosphofructokinase and pyruvate kinase were increased 1.7 to 3-fold in liver and/or adipose tissue by selenate treatment as compared to mice on selenium deficiency and mice with selenite administration. In contrast selenate treatment (SeVI) repressed the activity of liver pyruvate carboxylase the first enzyme in gluconeogenesis by about 33% in comparison to the selenium deficient (0Se) and selenite treated mice (SeIV). However the current study revealed an insulinomimetic role for selenate (selenium VI) also in type II diabetic animals due to a melioration of insulin resistance. In contrast selenium deficiency and especially selenite (selenium IV) impaired the diabetic status of dbdb mice, demonstrating the need for investigations on the insulinomimetic action of selenium due to the metabolism of different selenium compounds.

Keywords: Selenium; Insulinomimetic properties; Type II diabetes

1. Introduction

Selenium is largely known to develop its biological activity as an integral part of functional selenoproteins. The incorporation of the trace element into the redox-active selenocysteine residue of glutathione peroxidases, iodothyronine deiodinases and thioredoxin reductases is the basis for the physiological abilities of these proteins concerning the detoxification of hydrogen peroxide and lipid hydroperoxides, the equilibration of thyroid hormone metabolites and the reduction of cellular disulfides and ascorbate, respectively [1–3].

Selenium from varying chemical entities is absorbed by different intestinal mechanisms and both the storage in diverse organs and the extent of incorporation by the co-translational mechanism into functional selenoproteins depend on the chemical form of selenium [4–8].

For selenate (selenium VI) a further interesting physiological aspect with regard to diabetes was found. In type I diabetic rats and in tissue cultures insulinomimetic properties have been shown to evolve from selenate (selenium VI). During 10 weeks of oral treatment with selenate via drinking water the elevated blood glucose levels in rats with streptozotocin induced diabetes I (IDDM) could be reduced by 50 to 80% as compared to untreated rats. Especially during oral glucose challenge tests the insulinomimetic properties of selenate became vitally important. Blood glucose response to an oral glucose challenge was 40 to 50% lower in selenate treated diabetic rats in comparison to untreated controls [9]. Comparable results for insulinomimetic properties of selenate were also reported for type I diabetic rats receiving a daily intraperitoneal selenate injection [10,11]. In the type I diabetic rat model not only was a

* Corresponding author.
E-mail address: andreas.s.mueller@agrar.uni-giessen.de (A.S. Mueller).

0955-2863/03/$ – see front matter
doi:10.1016/j.nutbio.2003.08.001

higher disappearance rate of glucose and an enhanced glucose tolerance the outcome of selenate treatment but also a correction of the abnormally expressed glycolytic and gluconeogenic marker enzymes glucokinase, pyruvate kinase, phosphoenolpyruvate carboxykinase was observed as a consequence of selenate administration, indicating the involvement of selenate in major insulin dependent signaling pathways [9]. Findings on an influence of selenate administration on the expression and the activity of glucose-6-phosphate dehydrogenase and fatty acid synthetase confirmed this hypothesis [12]. Different results were found from experiments in rat adipocytes and rat hepatocytes on the cellular events triggered by selenate treatment as the cause of the insulinomimetic properties. In rat adipocytes treatment of the cells with selenate alone led to an increase in the phosphorylation of cAMP phosphodiesterase, S6 kinase and 210-, 170-, 120-, 95-, and 60 kDa proteins, whereas phosphorylation of the insulin receptor was not affected [13]. In a study with rat hepatocytes selenate could be demonstrated to enhance the phosphorylation of the β-subunit of the insulin receptor and of IRS1. Moreover in this trial the phosphorylation of the p42 and the p44 subunits of MAP kinase was raised by treatment of the hepatocytes with 500 μM selenate [14]. In conclusion the enhanced phosphorylation of diverse cellular proteins is believed to be responsible for an elevated translocation of glucose transporters, an increased glucose uptake and a modified gene expression of metabolic enzymes [15].

In the literature no information is available so far on a possible insulinomimetic role of selenate in animals with type II diabetes (NIDDM). Further no investigations on a distinct differentiation of the insulinomimetic properties of selenate in comparison to other selenium derivatives on glucose metabolism in diabetic animal models could be found.

The purpose of the present study was to investigate possible insulinomimetic properties of selenate in C57BL/KsOlaHsd-Leprdb mice with a defective leptin receptor, featuring severe symptoms of NIDDM such as hyperglycaemia, hyperinsulinaemia and high resistance to insulin [16,17]. Further the present study examines whether insulinomimetic properties are only derived from selenate or if other selenium compounds like selenite which are often used as selenium supplements for diets of laboratory animals also have insulinomimetic effects.

2. Materials and methods

2.1. Animals and experimental design

21 adult male dbdb mice (obtained from Harlan/Winkelmann), weighing 45.8 ± 1.57 g, individually housed in plastic cages with shavings as bedding material at 22°C, 12h:12h light dark cycle and fed a standard chow (Altromin 1320) containing 0.25 mg selenium as sodium selenite per kilogram diet, were put on a Se deficient diet (<0.03 mg Se/kg diet) based on torula yeast (Table 1). Except for the low Se content the Se deficient diet was formulated in accordance with the current NRC recommendations for mice [18]. The animals were randomly assigned to 3 groups of 7 animals each (group 0Se, group SeIV and group SeVI). Group 0Se was kept on selenium deficiency for 10 weeks and served to examine of the development of glucose tolerance and insulin resistance in type II diabetes during an alimentary selenium deficiency. Mice of the groups SeIV and SeVI were also fed the Se deficient diet over the 10 week experimental period but additionally these animals were supplemented with a daily dose of the +IV-selenium-derivative sodium selenite (group SeIV) or of the +VI-selenium-derivative sodium selenate (group SeVI) equivalent to 15% of their individual LD_{50} of both selenium

Table 1
Composition of the selenium deficient basal diet (<0.03 mg selenium/kg) for dbdb mice

Dietary components	Content (g/kg diet)
Torula yeast	300.0
Cellulose FTC 200	50.0
Glucose	50.0
Sucrose	50.0
Soybean oil	25.0
Coconut oil	25.0
DL-Methionin	3.0
Premix of minerals and trace elements (without selenium) [1]	66.6
Premix of vitamins [2]	10.0
Choline chloride	2.0
Maize grits	209.2
Maize meal	209.2
Total	**1000**

[1] Minerals and trace elements added per kg diet:
$CaCO_3$: 12.5 g = 5.090 mg Ca/kg diet
KH_2PO_4: 15.0 g = 2.650 mg P/kg diet
Na_2HPO_4: 7.5 g = 1.630 mg P/kg diet
$MgSO_4 \times 7\ H_2O$: 5.0 g = 508 mg Mg/kg diet
NaCl: 4.0 g = 1.56 g Na/kg diet
$CuSO_4 \times 5\ H_2O$: 20 mg = 5.10 mg Cu/kg diet
$FeSO_4 \times 7\ H_2O$: 250 mg = 50.2 mg Fe/kg diet
$ZnSO_4 \times H_2O$: 150 mg = 34.1 mg Zn/kg diet
$MnSO_4 \times H_2O$: 130 mg = 47.4 mg Mn/kg diet
$CrCl_3$: 7.5 mg = 2.47 mg Cr/kg diet
NaF: 2.2 mg = 0.99 mg F/kg diet
KJ: 0.3 mg = 0.25 mg J/kg diet
$CoSO_4 \times 7\ H_2O$: 1.2 mg = 0.25 mg Co/kg diet
$Na_2MoO_4 \times 2\ H_2O$: 0.5 mg = 0.2 mg Mo/kg diet
[2] Vitamins added per kg diet:
Vitamin A: 15.000 I.U.
Vitamin D: 1.500 I.U.
Vitamin E: 50 I.U.
Vitamin K_3: 5 mg
Vitamin B_1: 10 mg
Vitamin B_2: 10 mg
Vitamin B_6: 10 mg
Vitamin B_{12}: 0.02 mg
Niacin: 50 mg
Pantothenic acid: 10 mg
Biotin: 0.3 mg
Vitamin C: 150 mg

compounds (LD_{50} of sodium selenite and sodium selenate for mice: 3250 to 3600 μg/kg body weight [19]. The aqueous solutions of sodium selenite (96 μg/mL) and sodium selenate (105 μg/mL) were administerd by tube feeding. Thus the mice of group SeIV represented animals obtaining a selenium rich standard chow. The daily selenite dose given corresponded to the 10-fold daily requirement and it was therefore far below the acute toxic level. The mice of group SeVI served to examine the insulinomimetic properties of doses of selenate below the acute toxic level for the treatment of type II diabetes. Except for the special feeding of sodium selenite in group SeIV and sodium selenate in group SeVI the mice of the three experimental groups had free access to the selenium deficient basal diet and water.

During the experiment the current diabetic status in mice of all experimental groups was monitored by assessment of their glucose tolerance (OGCT) and their resistance to insulin (IR) before subjecting the mice to specified dietary conditions (initial status) and after 4, 6, 8 and 10 weeks of special feeding. The activities of glycolytic and gluconeogenic marker enzymes in the liver, hind limb muscle and adipose tissue served as parameters of the final diabetic status. Development of selenium status during the experiment was determined by measurement of GPx3 activity in plasma prepared before subjecting the mice to specified dietary conditions (initial status) and after 4, 6, 8 and 10 weeks of special feeding. The final selenium status of the mice was assayed by measurement of GPx1 activity in the liver and hind limb muscle.

All experimental procedures were approved by the Animal Care Authorities of Heidelberg University.

2.2. Performance of oral glucose challenge tests (OGCT) and test of insulin resistance (IR)

Oral glucose challenge tests (OGCT) were performed in mice fasted overnight. Therefore 2 g glucose per kg body weight were given to the mice by tube feeding using an aqueous glucose solution (100 mg D[+] glucose/mL). Glucose concentration was registered in blood samples taken from the tail vein before the glucose challenge and 20, 40, 60, 90, 120, 180, and 240 min after glucose administration.

Insulin resistance (IR) in mice fasted overnight was tested by subcutaneous injection of 2 I.U. insulin/kg body weight (Insuman ® Infusat 100 I.U./mL from AVENTIS Pharma Deutschland GmbH, Frankfurt/Main). Glucose concentration in blood sampled from the tail vein was recorded before starting the test and 30, 60, 90, 120, 180 and 240 min after insulin injection.

2.3. Analytical methods

2.3.1. Collection of samples and tissue preparation

During OGCT and IR blood from tail vein was sampled in heparinized hematocrit capillaries and glucose concentration was immediately determined.

Plasma for the determination of GPx3 activity was separated by sampling blood from the tail vein in heparinized hematocrit capillaries and centrifugation at 7.500*g* for 10 min.

After 10 weeks of special feeding the mice of the experimental groups 0Se, SeIV and SeVI were anesthesized in a carbon dioxide atmosphere and subsequently killed by decapitation. Liver, hind limb muscle and adipose tissue were removed immediately and 1:5 (w/v) homogenates of the above mentioned tissues were instantly prepared in 20 mM TRIS-HCl, 1 mM EDTA, pH 7.4 using a glass-glass homogenizer.

2.3.2. Enzymatic determinations

2.3.2.1. Determination of glucose concentration: Glucose concentration in blood samples was measured enzymatically using the glucose dehydrogenase assay [20].

2.4. Assessment of selenium status by determination of cellular glutathione peroxidase activity (GPx1) and activity of plasma glutathione peroxidase (GPx3)

Activity of GPx1 in the 10.000*g* cytosolic supernatant of crude homogenates from the liver and hind limb muscle and activity of GPx 3 in blood plasma were estimated by the indirect spectrophotometric procedure coupled to glutathione reductase [21]. NADPH oxidation was recorded for 5 min at 340 nm. A blank without added plasma or cytosolic supernatant was carried out for each sample. Activities of GPx1 and GPx3 were calculated from the absorption difference of both determinations. One unit of GPx1 and GPx3 activity was defined as one micromole NADPH oxidized per minute under the described conditions.

2.5. Assessment of the final diabetic status by the determination of marker enzymes of glycolysis (hexokinase, phophofructokinase, pyruvate kinase) and gluconeogenesis (glucose-6-phosphatase, fructose-1,6-diphosphatase, pyruvate carboxylase)

The activity of the glycolytic marker enzymes (hexokinase, phosphofructokinase, pyruvate kinase) and of the gluconeogenic marker enzymes (glucose-6-phosphatase, fructose-1,6-diphosphatase, pyruvate carboxylase) was measured photometrically by standard assays coupled to NAD/NADP – NADH/NADPH [22–27].

2.6. Determination of the selenium concentration in the basal diet

The selenium concentration in the selenium deficient basal diet was determined by Hydride Generation Atomic Absorption at the Institute of Animal Nutrition and Nutrition Physiology of the Justus Liebig University, Giessen.

Certified samples of compound feed (Mischfutter En-

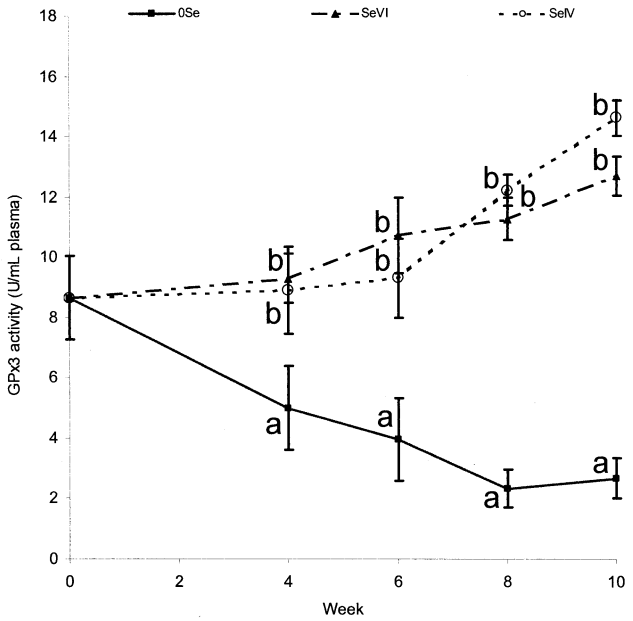


Fig. 1. Significant differences ($p<0.05$, Tukey test) between groups are indicated by different small letters. Each data point represents the mean±SD of 7 mice per group.

quete of the VDLUFA) served as reference material for selenium determination [28,29].

2.6.1. Statistical analysis

Statistical analysis of the experimental data was performed using the statistical package "SPSS 8.0 for Windows". A one way analysis of variance (ANOVA) was performed after ascertainment of the normality of distribution (Kolmogorov-Smirnov-Test or Shapiro-Wilk-Test) and the homogeneity of variance (Levene-Test) of the experimental data. If both conditions were fulfilled differences between means were evaluated using the Tukey-Test. If homogeneity of variance could not be ensured differences between means were examined using the Dunnett-T3-Test. Differences between means were assumed as significant at an error probability less than 5% ($P < 0.05$).

3. Results

During the experiment mice of all experimental groups lost body weight. The final body weights of the mice were 40.4 ± 2.65 g (group 0Se), 38.6 ± 3.10 g (group SeIV) and 39.6 ± 3.63 (group SeVI).

A differential development of plasma glutathione peroxidase activity (GPx3) was measured as a consequence of the diverse dietary conditions (Fig. 1). Starting from a mean activity of 8.65 ± 1.39 U/mL GPx3 activity consistently decreased in the selenium deficient mice of group 0Se to a final value of 2.68 ± 0.38 U/mL at week 10, whereas a continuous rise of GPx3 activity to final values of 14.6 ± 0.59 and 12.7 ± 0.66 was measured in the selenium treated mice of the groups SeIV and SeVI, respectively. Significant differences in GPx3 activity ($P < 0.05$) between the selenium deficient mice and the selenium treated mice of groups SeIV and SeVI already occurred after 4 weeks under the various experimental conditions.

Table 2
Activity of GPx1 (mU/mg protein) in the liver and in the hind limb muscle of dbdb mice kept on selenium deficiency for 10 weeks (0Se) and of dbdb mice treated with selenite (SeIV) or selenate (SeVI) for 10 weeks

Group Organ	0Se	SeIV	SeVI
Liver	396 ± 139[a]	1741 ± 205[b]	1599 ± 129[b]
Hind limb muscle	26.8 ± 3.87[a]	49.7 ± 10.2[b]	56.2 ± 12.4[b]

Significant differences ($p < 0.05$, Turkey test / Dunett-T3 test) between groups are indicated by different superscripts within a line.

Selenium deficiency and treatment with selenite or selenate were also reflected by the activity of cellular glutathione peroxidase (GPx1) in the liver and in the hind limb muscle of the mice (Table 2). After 10 weeks of selenium deficiency GPx1 activity in the liver was reduced to 23% and 25% as compared to the values measured in mice treated with selenite and selenate for 10 weeks. Likewise in the hind limb muscle of selenium deficient mice of group 0Se, GPx1 activity was decreased to about 54% and 48% in comparison with selenium supplied mice of groups SeIV and SeVI.

Fig. 2A summarizes how glucose tolerance of dbdb mice in the three experimental groups was affected after 10 weeks under the various dietary conditions in comparison to the initial status.

Mice of the initial status and of the three experimental groups exhibited exceedingly high fasting blood glucose concentrations (24.9 ± 0.7 mmol/L) which are typical for diabetic dbdb mice.

The extreme peak values in blood glucose concentration obtained 20 and 40 min after glucose administration which were already observed for the initial status group were significantly exceeded in mice kept on selenium deficiency for 10 weeks (0Se) and in mice treated with selenite for 10 weeks (SeIV). In contrast to this observation in mice treated with selenate for 10 weeks the peak values registered 20 and 40 min after the glucose challenge were slightly lower when compared to the initial status and significantly lower in comparison to selenium deficient mice and to mice treated with selenite. After a glucose challenge, recurrence of blood glucose concentration to the fasting level in mice with selenate administration for 10 weeks was comparably as fast as in the initial status, whereas the recovery from a glucose challenge in selenite treated mice and in selenium deficient mice was distinctly delayed. Thus in selenium deficient mice blood glucose concentration 240 min after a glucose challenge remained 41% above the fasting level. In mice treated with selenite for 10 weeks blood glucose concentra-

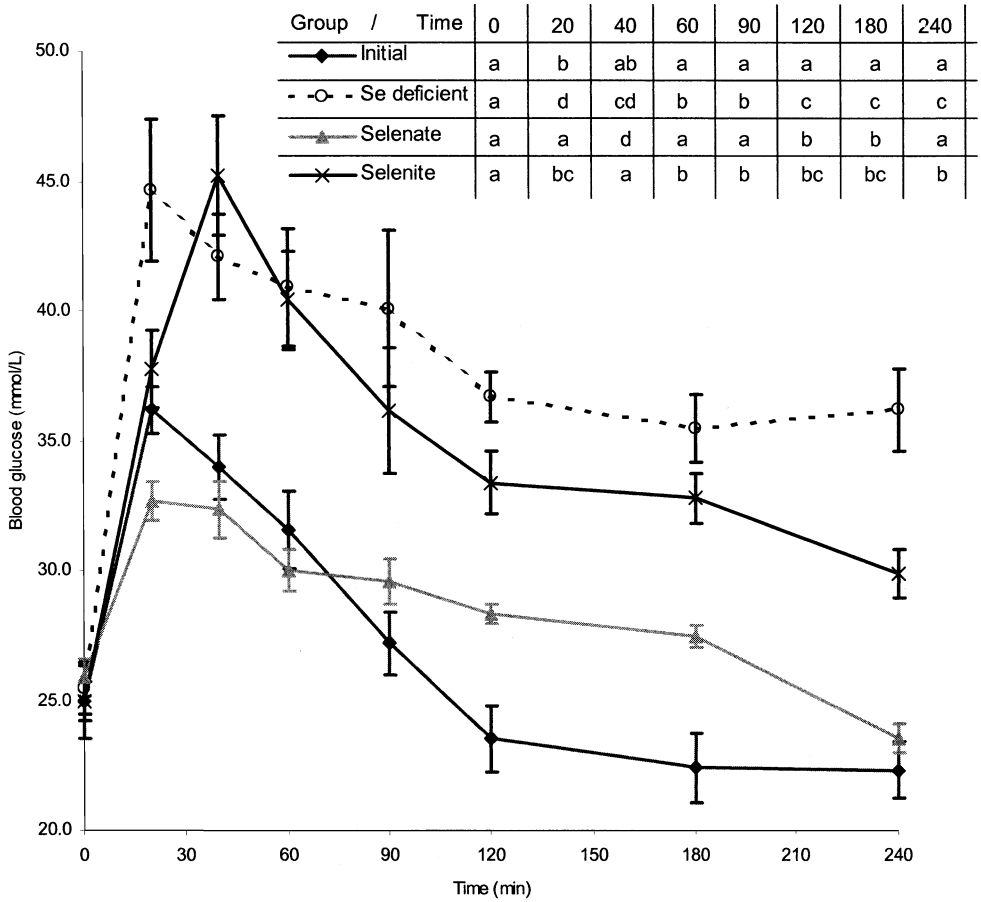


Fig. 2A. Significant differences ($p<0.05$, Tukey test/Dunnett-T3 test) between groups during OGCT are indicated by different small letters in the legend table. Each data point represents the mean ± SEM of 7 mice per group.

tion 4 hr after glucose administration even exceeded the fasting value by about 20%.

Fig. 2B compares the impact of 10 weeks of varying dietary conditions on insulin resistance in dbdb mice. The fasting blood glucose concentration prior to IR (0 min value: 24.5 ± 1.6 mmol/L) between the initial status and the three experimental groups did not differ significantly. 10 weeks of selenium deficiency (0Se) clearly diminished the properties of insulin. On the one hand the acute diminishing effect of an insulin challenge on blood glucose concentration (30 min: 3% reduction of the fasting blood glucose concentration, 60 min: 55%, 90 min: 65%) was comparably as strong as in the initial status, but on the other hand the return of blood glucose concentration towards the fasting level was significantly faster in the selenium deficient mice (120 min: 48% reduction of the fasting blood glucose concentration, 180 min: 15%) than in mice of the initial status (120 min: 52%, 180 min: 46%, 240 min: 35%). 240 min after the insulin challenge in mice kept on selenium deficiency for 10 weeks the fasting glucose value was even exceeded by 2%. Unexpectedly the daily administration of selenite (SeIV) for 10 weeks caused the most distinct impairment of insulin action associated with a markedly increased insulin resistance. 30, 60 and 90 min after the insulin challenge the reduction of the fasting blood glucose concentration was only 10%, 28% and 32%, respectively. Thus the acute reducing activity of insulin on blood glucose concentration in mice treated with selenite for 10 weeks was only one half of that obtained in mice of the initial status and in selenium deficient mice. Moreover the return of the blood glucose concentration to the fasting level was most rapid. 120 min and 180 min after the insulin challenge the reduction of the fasting level was only 16% and 6%. 240 min after the challenge the original fasting level was even exceeded by about 22%. Dbdb mice treated with the insulinomimetic selenium derivative selenate for 10 weeks featured the highest response to an insulin challenge. The reduction of the fasting blood glucose level in selenate treated mice was most distinct (30 min: 33% reduction of the fasting blood glucose concentration, 60 min: 62%, 90 min: 75%). Furthermore the insulin performance was significantly prolonged by selenate treatment and the return towards the fasting level was extremely slow (120 min: 82%, 180 min: 77%, 240 min: 62%). Insulin is involved in the gene expression of glycolytic and gluconeogenic marker enzymes. Therefore the relative insulin deficiency in type II

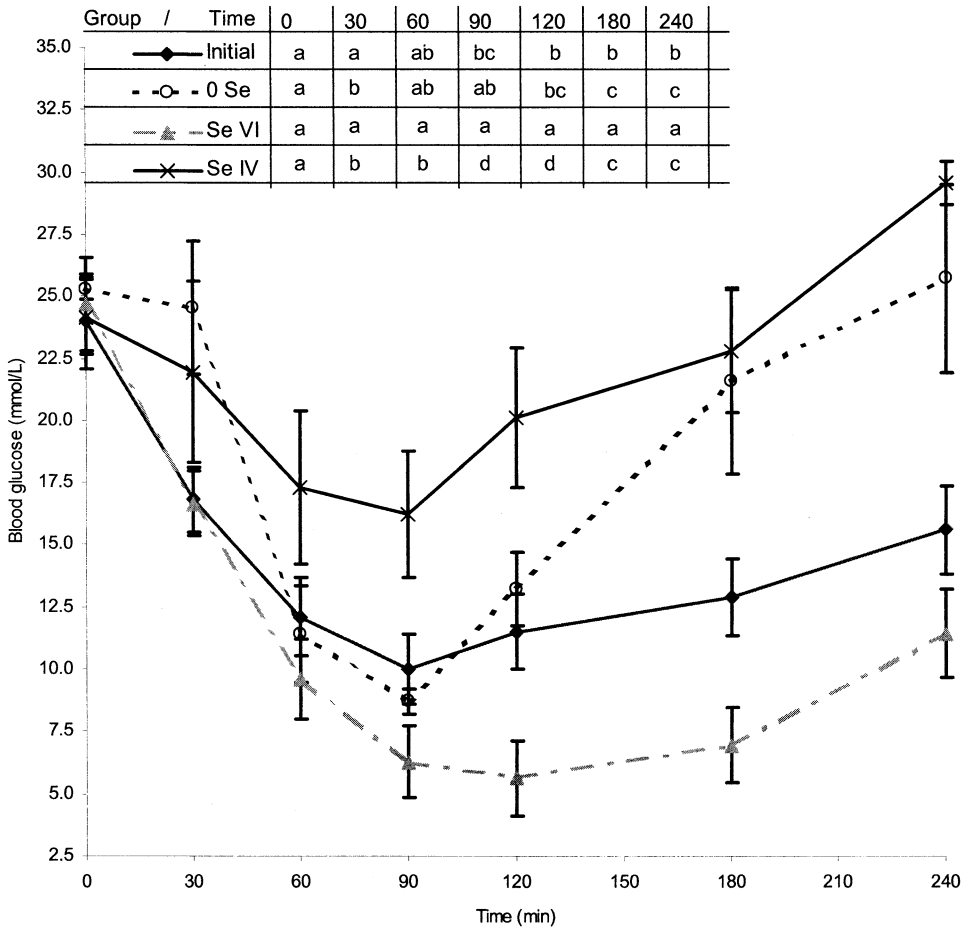


Fig. 2B. Significant differences ($p<0.05$, Tukey test/Dunnett-T3 test) between groups during IR are indicated by different small letters in the legend table. Each data point represents the mean ± SEM of 7 mice per group.

diabetes leads to a repression of glycolytic marker enzymes and to an enhanced expression of gluconeogenic marker enzymes. Thus in the present study the altered sensitivity to insulin was also reflected by the activity of some glycolytic and gluconeogenic marker enzymes in various tissues of the dbdb mice (Table 3). Selenate treatment led to a 3-fold increase in liver hexokinase activity as compared to selenium deficient and selenite treated mice. The activity of phosphofructokinase in liver and adipose tissue was elevated 2-fold and 1.7-fold in selenate treated mice in com-

Table 3
Activity of glycolytic and gluconeogenic marker enzymes (U/mg protein) in various tissues of dbdb mice kept on selenium deficiency for 10 weeks or treated with selenite (Se IV) or selenate (Se VI) for 10 weeks

Glycolytic/glyconeogenic marker enzyme	Organ	0 Se	Se IV	Se VI
Hexokinase	• Liver	0.08 ± 0.05^{a}	0.07 ± 0.05^{a}	0.26 ± 0.06^{b}
Phosphofructokinase	• Liver	9.25 ± 2.74^{a}	12.2 ± 0.51^{a}	18.5 ± 1.22^{b}
	• Skeletal muscle	5.61 ± 0.86^{b}	3.13 ± 0.86^{b}	4.74 ± 1.20^{ab}
	• Adipose tissue	0.22 ± 0.07^{a}	0.19 ± 0.08^{a}	0.38 ± 0.07^{b}
Pyruvate kinase	• Liver	5.20 ± 1.58^{a}	5.21 ± 0.69^{a}	6.25 ± 0.84^{a}
	• Skeletal muscle	13.0 ± 1.67^{a}	9.29 ± 1.19^{a}	10.8 ± 2.45^{a}
	• Adipose tissue	0.12 ± 0.05^{a}	0.09 ± 0.03^{a}	0.21 ± 0.03^{b}
Glucose-6-phosphatase	• Liver	2.62 ± 0.92^{a}	3.60 ± 0.72^{a}	3.89 ± 0.51^{a}
Fructose-1,6-bisphosphatase	• Liver	0.65 ± 0.24^{a}	1.00 ± 0.36^{a}	0.99 ± 0.38^{a}
Pyruvate carboxylase	• Liver	90.9 ± 16.8^{b}	103 ± 14.9^{b}	66.3 ± 11.6^{a}

Significant differences ($p < 0.05$, Tukey test / Dunnett-T3 test) between groups are indicated by different superscripts within a line.

parison to their selenium deficient and selenite treated companions. Within the glycolytic marker enzymes a 2-fold higher activity of pyruvate kinase was measured in the adipose tissue of selenate treated mice as compared to selenium deficient and selenite treated mice. In contrast to the observations for the above mentioned glycolytic enzymes 10 weeks of selenate treatment repressed the activity of liver pyruvate carboxylase the first enzyme in gluconeogenesis by the factor 1.5 in comparison to selenium deficient and selenite treated dbdb mice.

4. Discussion

4.1. Animal performance

A loss of body weight during the experiment (6.25 ± 1.82 g) occurred in all experimental groups and therefore could not be attributed to selenium deficiency or treatment with selenite and/or selenate. This fact is important in order to compare the diabetic status of the mice and to distinguish between genuine effects of the different dietary conditions and effects secondary to a reduction of body weight [9]. Possibly the changeover to the torula yeast diet was responsible for the weight reduction, although the diet contained sufficient amounts of gross energy (19.5 ± 1.31 MJ/kg diet) and crude protein (16.2 ± 1.24 g/100g diet) according to the recommendations [18].

4.2. Parameters of selenium status

During the experiment an efficient selenium depletion in group 0Se and a further improvement of selenium status in groups SeIV and SeVI, according to the experimental design, was reflected by the development of GPx3 activity in the plasma. Comparable results for the extent of loss of GPx3 activity during a 13 week selenium depletion period were reported for conventional black 6 mice and GPx1 knock out mice [30,31]. Since plasma glutathione peroxidase (GPx3) is synthesized predominantly in kidney, liver and lung its activity therefore provides evidence of the selenium status in these organs. Selenium deficiency leads to an immediate decrease in GPx3 expression and vice versa a rapid saturation in GPx3 expression is attained by selenium replenishment and continuous selenium administration [30]. In the present study the useful role for GPx3 activity as a sensitive parameter of the current body selenium status could be confirmed [29]. At the end of the experiment the efficacy of treatment under the various dietary conditions on selenium status was also reflected by the activity of cellular glutathione peroxidase in the liver and hind limb muscle. Comparable results for GPx1 activity in the liver (approx. 300 mU/mg protein) and hind limb muscle (approx. 20 mU/mg protein) were reported in a trial with mice kept on selenium deficiency for 8 weeks in comparison to mice with overexpression of GPx1 activity or mice fed with a diet containing 0.51 mg selenium/kg diet (GPx1 in the liver: approx. 1100 mU/mg protein, GPx1 in hind limb muscle: approx. 60 mU/mg protein) [32]. In a further report on the necessity of selenium supplementation for mice in addition to vitamin E supplementation comparable values for GPx1 activity in diverse tissues of mice were achieved [33].

4.3. Influences of selenium deficiency and administration of selenate and selenite on glucose tolerance and insulin resistance

Hitherto investigations on in vivo insulinomimetic properties of selenate were made exclusively in streptozotocin treated type I diabetic rats and in tissue cultures of hepatocytes and adipocytes. In the above mentioned studies in type I diabetic rats very high daily selenate doses close to the LD_{50} (3.5 mg/kg body weight x day [10], 3.2 mg/kg body weight x day [9], 4.5 mg/kg body weight x day [11]) were applied orally or by intraperitoneal injection to obtain a melioration of the diabetic status. Thereby type I diabetic streptozotocin treated rats show the following characteristics of IDDM:

- Markedly reduced insulin production with maximum levels of 20.0 ± 3.00 μU/mL [10,11,34]
- High starved blood glucose concentrations in the range of 15.0 ± 5.0 mmol/L [10,11,34]
- Low glucose tolerance: Recovery from a glucose challenge is distinctly delayed [10,11]
- High sensitivity to insulin: Insulin treatment reverses the diabetic symptoms completely [10,11]

The present study differs in three major points from previous investigations in rats:

1. Dbdb mice were used as a type II diabetic animal model displaying the following typical symptoms of NIDDM:
   - Massive obesity [17,35]
   - High starved glucose levels in adult animals in the range of 25.0 ± 5.0 mmol/L ([17,35], current study)
   - Low glucose tolerance: after a glucose challenge recurrence of blood glucose concentration to the initial value is noticeably delayed
   - High basal insulin levels (394 to 698 μU/mL) and pronounced insulin resistance: very high insulin doses are needed to reduce blood glucose concentration [17]
2. On account of the tremendous insulin secretion in dbdb mice, selenate treatment in the present study was carried out with lower doses of the selenium compounds to examine insulinomimetic properties (15% of the LD_{50}: 0.52 mg selenate or selenite/kg body weight x day). Lower selenium doses were further employed in order to check the practicability of selenium treatment in type II diabetes with regard to the toxicity of selenium compounds.

3. In the present study the insulinomimetic properties of selenate were examined in relation to the effects of selenium deficiency and selenite treatment on the diabetic status of dbdb mice.

The diabetic status of dbdb mice is subject to permanent aggravation during their lives [35]. With regard to their glucose tolerance the present study could prove a distinct insulinomimetic effect of selenate in type II diabetic dbdb mice in comparison to selenium deficient animals and mice treated with the frequently used feed additive selenite. In comparison to the initial status the present data suggest an insulinomimetic role for selenate concerning the advanced age of the selenate treated mice.

The insulinomimetic role of selenate due to an improvement of glucose tolerance after a glucose challenge is in accordance with previous studies in type I diabetic rats.

In contrast to reports from studies with type I diabetic rats [9–11] in the present study no lowering effect of selenate treatment on the starved blood glucose concentration, obtained prior to the glucose tolerance tests and the insulin resistance tests, could be observed. Possibly the advanced diabetic state of the dbdb mice, indicated by 2-fold higher starved blood glucose levels in comparison to the rats in the above mentioned studies and the distinctly lower daily selenate dose used in the current study are responsible for this fact.

In comparison to the studies in type I diabetic rats in the present study with type II diabetic dbdb mice the insulin resistance of the animals was checked in addition to their glucose tolerance. Thereby selenate treatment meliorated insulin resistance of dbdb mice in contrast to their selenium deficient and selenite treated companions, indicated by a more intensive and prolonged effect of a defined single insulin dose. Thus selenate was proved as acting as an insulin sensitizing agent in type II diabetic animals. It can be speculated that two independent physiological mechanisms are involved in the insulin sensitizing properties of selenate. In a trial with type I diabetic rats the selenate treated non diabetic control animals showed a lowered insulin release in response to a glucose challenge, suggesting for the current model that selenate on the one hand helps to break through the insulin resistance by a downregulation of the immense pancreatic insulin production in dbdb mice. On the other hand selenate was demonstrated to evolve a direct insulinlike effect by stimulating phosphorylation reactions of the β-subunit of the insulin receptor and other downstream components of the insulin signaling pathway like IRS 1, IRS 2, S6 kinase and MAPK [13,14,36]. Downstream the insulin receptor substrates 1 and 2 insulin signaling spreads into three pathways. The RAS-RAF-MEK-MAPK pathway triggers the expression of GLUT 3. The activation of the protein kinase B pathway is involved in the regulation of GLUT 1 synthesis and GLUT 4 translocation, while the activation of PI3 kinase is the second main stimulus for GLUT 4 activation [37,38]. As a whole these processes effect a stimulation of glucose uptake, especially in muscle and adipose tissue by an enhanced recruitment of the GLUT 4 transporter. In the current type II diabetic model the hypothesis of selenate intervention in the insulin secretory process and insulin mimicking processes is underlined by the unexpected adverse effect of selenite treatment on insulin resistance. 6 weeks of selenite administration (2.5 mg/kg body weight) to mice, made type I diabetic with streptozotocin led to a virtually complete normalization of plasma glucose levels and a melioration of glucose tolerance due to a glucose challenge. These effects were observed as a consequence of a nearly complete restoration of the beta cells and a normalization of insulin secretion to levels observed in non diabetic control mice. Thereby the restoration of beta cell mass was explained by the antioxidative effects of selenite treatment [39]. Otherwise no investigations on direct insulin mimicking effects of selenite as a consequence of enhanced cellular phosphorylation signals in peripheral tissues could be found in the literature.

For the current type II diabetic model the application of this hypothesis means that on the one hand the tremendous pancreatic insulin secretion is augmented by selenite treatment. But on the other hand the vicious circle of peripheral insulin resistance is stimulated by selenite treatment, assuming that selenite possesses no peripheral insulin mimicking properties.

Recently several new hypotheses have been discussed as being responsible for peripheral insulin resistance.

In obese rodents the enhanced expression of the protein resistin in adipose tissue is discussed as being an important factor of peripheral insulin resistance [40]. Treatment with thiazolidinediones a class of antidiabetic drugs led to a marked downregulation of resistin and a melioration of glucose tolerance and insulin resistance via the nuclear PPARγ receptor [41]. The exact mechanism of resistin action is not yet understood.

Better understood mechanisms of insulin resistance suggest a weakening of the phosphorylation reactions of the insulin signaling pathway as being responsible for peripheral insulin resistance [42].

Thereby enhanced activities of several protein phosphatases like protein tyrosine phosphatase 1B (PTP1B) or SH containing inositol phosphatase 2 (SHIP2) are discussed as being responsible factors impairing insulin signaling at different levels of the insulin signaling pathway [43–45]. In addition to previous investigations on insulinomimetic properties of selenate which concentrated on the examination of phosphorylated cellular compounds it would be recommendable to examine if enhancement of cellular phosphorylation reactions by selenate and melioration of insulin resistance in the current type II diabetic model could be attributed to an inhibition of protein tyrosine phospha-

tases as recently demonstrated for vanadate another trace element with insulinomimetic properties [46,47].

4.4. Influences of selenium deficiency and administration of selenate and selenite on the activity of glycolytic and gluconeogenic key enzymes

Metabolic abnormalities of glucose metabolism in genetically obese dbdb mice begin to develop at an average age of 4 weeks. Onset of massive obesity occurs in combination with an abnormally high insulin production and hyperglycaemia. These processes lead to severe changes especially in hepatic glycolysis and gluconeogenesis. Initially the activities of both pathways show enhanced activities. With increasing age and elevated insulin resistance enzyme activities of the glycolytic pathway tend to decrease in relation to gluconeogenic marker enzymes which are accented. In the current study for some glycolytic and gluconeogenic marker enzymes changes in their activity in various tissues were achieved. 10 weeks of selenate treatment led to an augmentation of the glycolytic pathway, indicated by enhanced activities of hexokinase in the liver, phosphofructokinase in the liver, skeletal muscle and adipose tissue and of pyruvate kinase in adipose tissue in comparison to the activities of these enzymes in selenium deficient mice and selenite treated mice. With regard to the gluconeogenic pathway a suppression of liver pyruvate carboxylase activity in the selenate treated dbdb mice was measured as compared to the selenium deficient and selenite treated mice.

Since the hormone insulin is involved in the enhancement of glucose transport in skeletal muscle and adipose tissue, the amelioration of glucose breakdown by enhancing the expression and activity of glycolytic enzymes in the liver, skeletal muscle and adipose tissue and the suppression of gluconeogenesis in the liver [38], the changes observed in the activities of the glycolytic and gluconeogenic enzymes could be interpreted as a consequence of the insulin sensitizing effect of selenate and of the lowered insulin resistance caused by selenate in the current type II diabetic animal model.

Comparable influences of selenate treatment on the expression of glycolytic and gluconeogenic marker enzymes were also described for type I diabetic rats. Streptozotocin treatment caused a 90% loss of pancreatic insulin secretion, a distinct downregulation of the liver glycolytic marker enzymes glucokinase and pyruvate kinase and a significant upregulation of the liver gluconeogenic marker enzyme phosphoenolpyruvate carboxykinase [9]. 10 weeks of oral selenate administration effected a restoration of the enzymes' activities to 40 to 65% of the values in non diabetic control rats [9]. Alternative treatment with selenate or vanadate was also reported to normalize the decreased expression and activity of glucose-6-phosphate dehydrogenase and fatty acid synthetase, two major enzymes of lipid metabolism [12].

4.5. Conclusions and future aspects

In conclusion the present study revealed insulinomimetic properties of selenate (selenium VI) also in type II diabetic animals, as indicated by enhanced glucose tolerance and changes in the activity of some major glycolytic and gluconeogenic marker enzymes.

In contrast selenium deficiency and especially selenite impaired insulin resistance and led to an aggravation of glucose tolerance and glucose metabolism.

Future investigations on the insulinomimetic properties of selenate in type II diabetic animals in comparison to other selenium compounds should be focused on the biochemical pathways of selenate and selenite [48] and their effects on reducing cellular thiols like glutathione. Long term lowered concentrations of GSH were demonstrated to enhance insulin sensitivity [49], whereas treatment with N-acetylcysteine reversed this effect [50]. Selenite is known to be reduced more rapidly than selenate by glutathione [48,51] suggesting that selenate is able to lower cellular GSH concentrations in insulin sensitive organs more sustainedly. Due to these facts perhaps there is an analogy to vanadate metabolism. In insulin resistant glutathione depleted adipocytes the reduction of vanadate (vanadium V) was delayed in comparison to the reduction of vanadyl (vanadium IV). Simultaneously vanadate (vanadium V) was demonstrated to be a stronger inhibitor of protein tyrosine phosphatase 1B, a negative regulator of insulin signaling [50].

If this hypothesis is applied to the effects observed for selenate two independent mechanisms are imaginable:

1. Selenate leads to a long term reduction of cellular thiols, especially reduced glutathione
2. Selenate acts as a strong inhibitor of protein tyrosine phosphatases

In addition to metabolic aspects of selenium compounds in the insulin sensitive organs the effect of different selenium compounds on pancreatic insulin production should be examined against the background of the concentration of reduced thiols [52]. In vivo studies in type II diabetic species or cultures of pancreatic beta cells would be convenient models for such examinations.

Acknowledgments

We thank AVENTIS Pharma Deutschland GmbH for supporting the study. In particular we thank Dr. A.W. Herling from AVENTIS Pharma Deutschland GmbH for the discussion of interesting aspects and his cooperation in this study.

References

[1] Brigelius-Flohé R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 1999;27:951–65.

[2] Köhrle J. Thyroid hormone deiodinases – a selenoenzyme family acting as gate keepers to thyroid hormone action. Acta Med Austriaca 1996;23:17–30.

[3] May JM, Mendiratta S, Hill KE, Burk RF. Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. J Biol Chem 1997;272:22607–10.

[4] Wolffram S, Ardüser F, Scharrer F. In vivo intestinal absorption of selenate and selenite by rats. J Nutr 1985;115:454–9.

[5] Wolffram S, Berger B, Grenacher B, Scharrer E. Transport of selenoamino acids and their sulfur analogues across the intestinal brush border membrane. J Nutr 1989;119:706–12.

[6] Behne D, Kyriakopoulos A, Scheid S, Gessner H. Effects of chemical form and dosage on the incorporation of selenium into tissue proteins. J Nutr 1991;121:806–14.

[7] Walczak R, Hubert N, Carbon P, Krol A. Solution structure of SECIS, the mRNA element required for eukaryotic selenocysteine insertion - interaction studies with the SECIS-binding protein SBP. Biomed Environ Sci 1997;10:177–81.

[8] Sunde RA, Evenson JK. Serine incorporation into the selenocysteine moiety of glutathione peroxidase. J Biol Chem 1987;262:933–7.

[9] Becker DJ, Reul B, Ozcelikay AT, Buchet JP, Henquin JC, Brichard SM. Oral selenate improves glucose homeostasis and partly reverses abnormal expression of liver glycolytic and gluconeogenic enzymes in diabetic rats. Diabetologia 1996;39:3–11.

[10] McNeill JH, Delgatty HL, Battell ML. Insulinlike effects of sodium selenate in streptozotocin-induced diabetic rats. Diabetes 1991;40: 1675–8.

[11] Battell ML, Delgatty HL, McNeill JH. Sodium selenate corrects glucose tolerance and heart function in STZ diabetic rats. Mol Cell Biochem 1998;179:27–34.

[12] Berg EA, Wu JY, Campbell L, Kagey M, Stapleton SR. Insulin-like effects of vanadate and selenate on the expression of glucose-6-phosphate dehydrogenase and fatty acid synthase in diabetic rats. Biochimie 1995;77:919–24.

[13] Hei YJ, Farahbakshian S, Chen X, Battell ML, McNeill JH. Stimulation of MAP kinase and S6 kinase by vanadium and selenium in rat adipocytes. Mol Cell Biochem 1998;178:367–75.

[14] Stapleton SR, Garlock GL, Foellmi-Adams L, Kletzien RE. Selenium potent stimulator of tyrosyl phosphorylation and activator of MAP kinase. Biochim Biophys Acta 1997;1355:259–60.

[15] Stapleton SR. Selenium: an insulin-mimetic. Cell Mol Life Sci 2000; 57:1874–9.

[16] Kodama H, Fujita M, Yamaguchi M. Development of hyperglycemia and insulin resistance in conscious genetically diabetic (C57BL/KsJ-dbdb) mice. Diabetologia 1994;37:739–44.

[17] Chua S, Liu MS, Li Q, Yang L, Thassanapaff VT, Fisher P. Differential beta cell responses-hyperglycaemia and insulin resistance in two novel congenic strains of diabetes (FVB-Leprdb) and DBA-Lepob) mice. Diabetologia 2002;45:976–90.

[18] National Research Council. Nutrient requirements of laboratory animals, 4th revised edition. Washington, DC: National Academy Press, 1995.

[19] Shibata Y, Morita M, Fuwa K. Selenium and arsenic in biology: their chemical forms and biological functions. Adv Biophys 1992;28:31–80.

[20] Stahl M, Brandslund I, Iversen S, Filtenborg JA. Quality assessment of blood glucose testing in general practitioners′ offices improves quality. Clin Chem 1997;43:1926–31.

[21] Tappel ME, Chaudiere J, Tappel AL. Glutathione peroxidase activities of animal tissues. Comp Biochem Physiol 1982;73(B):945–9.

[22] Ureta T, Bravo R, Babul J. Rat liver hexokinase during development. Enzyme 1975;20:334–48.

[23] Uyeda K. Studies on the reaction mechanism of skeletal muscle phosphofructokinase. J Biol Chem 1970;245:2268–75.

[24] Blair JB, Cimbala MA, Foster JL, Morgan RA. Hepatic pyruvate kinase. J Biol Chem 1976;25:3756–62.

[25] Allegre M, Ciudad CJ, Fillat C, Guinovart JJ. Determination of glucose-6-phospahatase activity using the glucose dehydrogenase coupled reaction. Anal Biochem 1988;173:185–9.

[26] Pontremoli S, Melloni E. Fructose-1.6-diphosphatase from rabbit liver. Methods Enzymol 1975;42:354–9.

[27] Warren GB, Tipton KF. Pig liver pyruvate carboxylase. Purification, properties and cation specifity. Biochem J 1976;139:297–310.

[28] Most, E, Pallauf, J. Bestimmung von Selen in biologischen Matrices mittels Hydrid- und Graphitrohr-AAS. VDLUFA-Kongress, Halle (Saale), book of abstracts 1999:158.

[29] Müller AS, Pallauf J, Most E. Parameters of dietary selenium and vitamin E deficiency in growing rabbits. J Trace Elem Med Biol 2002;16:47–55.

[30] Schwaab V, Faure J, Dufaure JP, Drevet JR. GPx3: The plasma-type glutathione peroxidase is expressed under androgenic control in the mouse epididymis and vas deferens. Mol Reprod Development 1998; 51:362–72.

[31] Cheng WH, Ho YS, Ross DA, Valentine BA, Combs GF jr, Lei XG. Cellular glutathione peroxidase knockout mice express normal levels of selenium-dependent plasma and phospholipid hydroperoxide glutathione peroxidases in various tissues. J Nutr 1997;127:1445–50.

[32] Cheng WH, Ho YS, Ross DA, Han Y, Combs GF, Lei XG. Overexpression of cellular glutathione peroxidase does not affect expression of plasma glutathione peroxidase or phospholipid hydroperoxide glutathione peroxidase in mice offered diets adequate or deficient in selenium. J Nutr 1997;127:675–80.

[33] Cheng WH, Valentine BA, Lei XG. High levels of dietary vitamin E do not replace cellular glutathione peroxidase in protecting mice from acute oxidative stress. J Nutr 1999;129:1951–7.

[34] Nishigaki A, Noma H, Kakizawa T. The relation between doses of streptozotocin and pathosis in induced diabetes mellitus. Shikwa Gakuho 1989;89:639–62.

[35] Chan TM, Young KM, Hutson NJ, Brumley FT, Exton JH. Hepatic metabolism of genetically diabetic (db/db) mice. I. Carbohydrate metabolism. Am J Physiol 1975;279:1702–12.

[36] Ezaki O. The insulin effects of selenate in rat adipocytes. J Biol Chem 1990;265:1124–8.

[37] Hall RK, Granner DK. Insulin regulates expression of metabolic genes through divergent signaling pathways. J Basic Clin Physiol Pharmacol 1999;10:119–33.

[38] Newsholme EA, Dimitriadis G. Integration of biochemical and physiologic effects of insulin on glucose metabolism. Exp Clin Endocrinol Diabetes 2001;109(Suppl.):122–34.

[39] Ghosh R, Mukherjee B, Chatterjee M. A novel effect of selenium on streptozotocin-induced diabetic mice. Diabetes Res 1994;25:165–71.

[40] Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, Enders GH, Silberg DG, Wen X, Wu GD, Lazar M. A family of tissue-specific resistin-like molecules. Proc Natl Acad Sci USA 2001; 98:502–6.

[41] Willson TM, Lambert MH, Kliewer SA. Peroxisome proliferator-activated receptor g and metabolic disease. Annu Rev Biochem 2001; 70:341–67.

[42] Shao J, Yamashita H, Qiao L, Friedman JE. Decreased Akt kinase activity and insulin resistance in C57BL/KsJ-Leprdb/db mice. J Endocrinol 2000;167:107–15.

[43] Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, Xie N, Wilcox D, Jacobson P, Frost I, Kroeger PE, Reilly RM, Koterski S, Opgenorth TJ, Ulrich RG, Crosby S, Butler M, Murray SF, McKay R, Bhanot S, Monia BP, Jirousek MR. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci 2002;99:11357–62.

[44] Mahadev K, Zilbering A, Zhu L, Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1B in vivo and enhances the early insulin action cascade. J Biol Chem 2001;276:21938–42.
[45] Hori H, Sasaoka T, Ishihara H, Wada T, Murakami S, Ishiki M, Kobayashi M. Association of SH2-containing inositol phosphatase 2 with the insulin resistance of diabetic db/db mice. Diabetes 2002;51:2387–94.
[46] Pugazhenti S, Tanha F, Dahl B, Khandelwal RL. Decrease in protein tyrosine phosphatase activities in vanadate-treated obese Zucker (fa/fa) rat liver. Mol Cell Biochem 1995;153:125–9.
[47] Tracey AS. Hydroxamido vanadates: aqueous chemistry and function in protein tyrosine phosphatases. J Inorg Biochem 2000;80:11–6.
[48] Shiobara Y, Ogra Y, Suzuki KT. Speciation of metabolites of selenate in rats by HPLC-ICP-MS. Analyst 1999;124:1237–41.
[49] Garant MJ, Kole S, Maksimova EM, Bernier M. Reversible change in thiol redox status of the insulin receptor β-subunit in intact cells. Biochemistry 1999;38:5896–904.
[50] Lu B, Ennis D, Lai R, Bogdanovic E, Nikolov R, Salamon L, Fantus C, Tien L, Fantus IG. Enhanced sensitivity of insulin-resistant adipocytes-vanadate is associated with oxidative stress and decreased reduction of vanadate (+5)-vanadyl (+4). J Biol Chem 2001;276:35589–98.
[51] Usami M, Tabata H, Ohno Y. Effects of glutathione depletion on selenite- and selenate-induced embryotoxicity in cultured rat embryos. Teratog Carcinog Mutagen 1999;19:257–66.
[52] Ammon HP, Abdel-Hamid M, Rao PG, Enz G. Thiol-dependent and non-thiol-dependent stimulations of insulin release. Diabetes 1984;33:251–7.

Journal of Animal Physiology and Animal Nutrition ISSN 0931-2439

ORIGINAL ARTICLE

Effects of a supranutritional dose of selenate compared with selenite on insulin sensitivity in type II diabetic dbdb mice

A. S. Müller, E. Most and J. Pallauf

Institute of Animal Nutrition and Nutritional Physiology, Justus Liebig University Giessen, Giessen, Germany

Correspondence
Josef Pallauf, Institute of Animal Nutrition and Nutritional Physiology, Heinrich-Buff-Ring 26–32, D-35392 Giessen, Germany.
Tel: +49 641 99 39230; Fax: +49 641 99 39239;
E-mail: josef.pallauf@ernaehrung.uni-giessen.de

Received: 15 September 2004;
accepted: 12 November 2004

Summary

The present study was performed to examine the mechanism by which selenate ameliorates the insulin sensitivity in type II diabetic dbdb mice. Therefore, 21-adult female dbdb mice were randomly assigned to three experimental groups (0Se, SeIV and SeVI) with seven animals per group. Mice of group 0Se were fed with a selenium-deficient diet (<0.02 mg Se/kg) based on wheat and torula yeast for 8 weeks whereas the mice of groups SeIV (selenite) and SeVI (selenate) were fed with sodium selenite and sodium selenate (up to 35% of the LD_{50} for mice in eighth week), in addition to the diet by daily tube feeding. Eight weeks of selenate application led to significantly elevated insulin sensitivity in comparison with selenium deficiency and selenite application. The activity of cytosolic protein tyrosine phosphatases (PTPs) as important negative regulators of insulin signalling was reduced from 53.8% to 22.5% in the liver and skeletal muscle of selenate-treated mice in comparison with the selenium deficient and selenite-treated controls, suggesting an inhibition of PTPs by intermediary selenate metabolites. In an additional *in vitro* inhibition study, selenate (oxidation state +VI) did not inhibit PTP activity. Selenium metabolites in the oxidation state +IV were found to be the actual inhibitors of PTP activity. In conclusion, the results of the present study show that one possible mechanism by which supranutritional selenate doses enhance insulin sensitivity in type II diabetic dbdb mice is based on the inhibition of PTPS as negative regulators of insulin signalling. Moreover the cellular metabolism of selenate including its intermediary reduction to the oxidation state +IV seems to play a crucial role during this process.

Introduction

The trace element selenium develops its biological functions through the redox-active selenocysteine residue as an integral part of glutathione peroxidases, iodothyronine deiodinases and thioredoxin reductases. Depending on the physiological state, supplementing 0.1–0.3 mg selenium/kg diet to laboratory, companion and farm animals is normally sufficient to achieve optimum activities for the above-mentioned selenoproteins, which are involved in the regulation of the antioxidative balance of tissues (Brigelius-Flohé, 1999), the equilibrium of the thyroid hormones T4 and T3 (Koehrle, 1996) and the reduction of cellular disulphides and ascorbate (May et al., 1997).

In animals, selenium from various chemical compounds is absorbed by different mechanisms and distributed to peripheral tissues in a specific manner. Selenomethionine is absorbed in the small intestine by the same mechanism as its sulphur analogue methionine. Selenate, representing the hexavalent selenium oxidation state, is absorbed unmodified either by a sodium symporter or by an anion

exchange mechanism, while selenite representing the tetravalent selenium oxidation state, might form selenotrisulphides and selenopersulphides from the reaction with thiols or cysteine prior to its absorption (Wolffram et al., 1985, 1989).

After uptake into tissues, selenium is reduced to the selenide oxidation state (Behne et al., 1991; Shiobara et al., 1999; Suzuki and Ogra, 2002), from which selenium can be incorporated into the selenocyteine residues of functional selenoproteins by a cotranslational mechanism using a modified serine tRNA (Sunde and Evenson, 1987; Walczak et al., 1997).

In recent years, a specific function has been investigated for selenate with regard to diabetes. The application of supranutritive doses of sodium selenate (up to the individual LD_{50}) to type I diabetic rats was found to ameliorate the diabetic status of the rats including the reduction of their enhanced blood glucose concentration and the normalization of abnormally expressed glycolytic and gluconeogenic marker enzymes (McNeill et al., 1991; Berg et al., 1995; Becker et al., 1996; Battell et al., 1998; Stapleton, 2000). Experiments with tissue cultures showed that an enhanced phosphorylation of the β-subunit of the insulin receptor and further downstream components of the insulin-signalling pathway are presumably responsible for the antidiabetic characteristics also referred to as insulinomimetic properties of selenate (Stapleton et al., 1997; Hei et al., 1998). In dbdb mice displaying symptoms of obesity and severe insulin-resistant type II diabetes, selenate treatment led to an enhanced insulin sensitivity and a modification in the activity of glycolytic and gluconeogenic marker enzymes, while selenium deficiency and selenite application did not alter the age-dependent diabetic status (Müller et al., 2003).

In particular, the results for insulin sensitivity led to the assumption that the inhibition of protein tyrosine phosphatases (PTPs) may play a key role with regard to the antidiabetic action of selenate.

In the present study with young female dbdb mice, the effect of a supranutritional dose of selenate compared with selenite and selenium deficiency on insulin sensitivity and the activity of protein tyrosine phosphatases was therefore examined.

Materials and methods

Experimental design and feeding experiment with dbdb mice

Twenty-one 6-week-old female dbdb mice (obtained from Harlan Winkelmann, Borchen, Germany) with a mean live weight of 43.7 ± 2.0 g were randomly assigned to three groups of seven animals each (0Se, selenium-deficient group, SeIV, selenite-treated group, SeVI, selenate-treated group). All groups received a peletted selenium-deficient diet (<0.02 mg Se/kg diet) containing 45% wheat, 30% torula yeast, 5% cellulose, 2.5% coconut oil, 2.5% soya bean oil, 6.7% mineral premix, 1.0% vitamin premix 0.3% DL-methionine, 0.2% choline chloride and 6.8% maize starch. With the exception of selenium (<0.02 mg/kg diet), the composition of the diet met the recommendations for mice (NRC, 1995) and was fed for 8 weeks.

The animals of the groups SeIV and SeVI were supplemented with weekly increasing doses of sodium selenite and sodium selenate (starting with 15% of the LD_{50} for mice and reaching 35% of the LD_{50} in eighth week, LD_{50} selenite and selenate = 3.5 mg/kg body weight) by tube feeding. During the experiment, the animals were kept individually in plastic cages with shavings as bedding material at 22 °C and a 12 h:12 h light:dark cycle. The animals had *ad libitum* access to the diet and bidistilled water.

Before subjecting the mice to the defined dietary conditions and after 8 weeks of special feeding, the glucose tolerance and the insulin sensitivity of the animals were tested. Feed consumption and body weight gain of the mice were recorded daily.

Performance insulin sensitivity tests

Insulin sensitivity in mice that fasted overnight was tested by subcutaneous injection of 2 I.U. insulin/kg body weight (Insuman® Infusat 100 I.U./ml; AVENTIS Pharma Deutschland GmbH, Frankfurt/Main Germany). Glucose concentration in blood, sampled from the tail vein was recorded before starting the test and 30, 60, 90, 120, 180 and 240 and 300 min after insulin injection.

For blood sampling from the tail vein, the tail tip was abscised and the blood flow was stimulated by gently massaging the tail. The blood was collected in heparinized haematocrit capillaries.

Analytical methods

Determination of the selenium concentration in the basal diet

The selenium concentration in the selenium-deficient diet was determined by hydride generation atomic absorption as described previously (Most and Pallauf, 1999; Müller et al., 2002). Certified samples

of compound feed (Ring test from the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten) served as reference material for selenium determination.

Assessment of the selenium status by measurement of plasma glutathione peroxidase and liver cellular glutathione peroxidase

The development of selenium status was determined by measurement of glutathione peroxidase (GPx3) before subjecting the mice to the specified dietary conditions and at the end of the experiment. The final selenium status was assayed by measurement of glutathione peroxidase (GPx1) activity in the liver. Activity of both glutathione peroxidases was measured using the indirect spectrophotometric procedure coupled with glutathione reductase (Tappel et al., 1982). NADPH oxidation was recorded for 3 min at 340 nm. A blank without adding plasma or cytosolic supernatant was carried out for each sample. Activities of GPx1 and GPx3 were calculated from the absorption difference. One unit of GPx1 or GPx3 activity was defined as 1 μmol of NADPH oxidized per minute under the described conditions. The activity of GPx1 was normalized to 1 mg protein.

Determination of the activity of cytosolic protein tyrosine phosphatases in the liver and skeletal muscle

Activity of PTPs was determined with modifications according to a method which is based on the hydrolysis of paranitrophenyl phosphate (pNPP) (Zhu and Goldstein, 2002). For the analysis of PTP activity, 1:5 (w/v) homogenates of liver and skeletal muscle were prepared in a buffer consisting of 50 mmol/l 4-(-2-hydroxyethylpiperazine-1-ethanesulphonic acid (HEPES), 50 mmol/l NaCl, 1 mmol/l EDTA and 0.1 mmol/l phenylmethylsulphonylfluoride (PMSF) with pH 6.0. The cytosolic fraction was obtained by centrifugation at 60 000 ***g*** for 30 min at 2 °C. Subsequently, 10 μl liver cytosol or 20 μl muscle cytosol were pre-incubated at 25 °C in 240 μl (liver) or 230 μl (skeletal muscle) of a buffer containing 50 mmol/l 2-morpholinoethanesulfonic acid (MES), 50 mmol/l NaCl, 2 mmol/l EDTA and 0.1 mmol/l PMSF with a pH of 6.5 for 3 min. In addition to 250 μl of the same buffer, 20 mmol/l of the substrate pNPP was added and the mixture was further incubated at 25 °C for 10 min (liver) or 15 min (muscle). The reaction was terminated by the addition of 500 μl 2 M NaOH and the absorption was read in a Beckman DU 64 spectrophotometer (Beckman, Fullerton, CA, USA) at a wavelength of 410 nm. A blank without cytosol was carried out for all determinations. The activity of PTPs was calculated using an extinction coefficient of 0.0166/μM/cm for the resulting paranitrophenolate ion and normalized to 1 mg protein.

In vitro *inhibition of cytosolic protein tyrosine phosphatases by selenium compounds in dbdb mouse liver*

The activity of PTPs was measured in pooled liver cytosol from three age-matched adult female dbdb mice fed with a standard chow containing 0.25 mg Se/kg diet. Cytosol was prepared as described for activity determination of PTPs. The activity of PTPs was assessed as described above for liver homogenates obtained from the *in vivo* trial. In addition to the above procedure, 10 μl of aqueous solutions of sodium selenate (oxidation state +IV), non-enzymatically reduced selenate (using 37% HCl as the reducing agent, oxidation state +IV), sodium selenite (oxidation state +IV), selenious acid (oxidation state +IV) and freshly synthesized selenotrisulphides from the reaction of reduced glutathione and selenite in a molar ratio of 4:1 (synthesized according to a standard protocol [Ganther, 1968; Self et al., 2000], oxidation state 0), reaching final selenium concentrations of 25–5000 μmol/l were added before incubating the reaction mixtures with pNPP for 10 min. The reaction was terminated by the addition of 500 μl of 2 M NaOH and the absorption was read in a Beckman DU 64 spectrophotometer at a wavelength of 410 nm. A blank without cytosol was carried out for all determinations. The activity of PTPs was calculated using an extinction coefficient of 0.0166/μM/cm for the resulting paranitrophenolate ion. The inhibition of PTPs was expressed as a percent inhibition in comparison with the PTP activity reached in liver cytosol without addition of selenium compounds.

Determination of protein content in homogenates of liver and skeletal muscle

The protein content of the cytosolic fraction of liver, kidney and skeletal muscle was determined using a standard method (Bradford, 1976).

Statistical analysis

Statistical analysis of the experimental data was performed using the statistical package 'SPSS 8.0 for Windows'. For the parameters glutathione peroxidase and protein tyrosine phosphatase a one-way analysis of variance (ANOVA) was performed. After ascertaining the normality of distribution (Kolmogorov–Smirnov test and Shapiro–Wilk test) and the homogeneity of variance (Levene test) of the

experimental data differences between means were evaluated using the Tukey test. Differences between mean values were assumed as significant at an error probability <5% ($p < 0.05$).

Differences between the mean values of blood glucose concentration for the single points in time during insulin sensitivity test (IST) were evaluated using the Dunnett T3 test as homogeneity of variance was partially not fulfilled.

Results

Animal performance, feed consumption and development of body weight

The course of the experiment was without complications and considering their adiposity caused by the genetic leptin receptor defect, the mice of all experimental groups were in good condition. One mouse in the selenate group died 8 h after the final insulin sensitivity test, possibly as a consequence of a long-lasting fall of the blood glucose concentration beyond a value of 2 mmol/l.

The average body weight of 43.7 ± 2.0 g with mean of 44.7 ± 2.5 in group 0Se, 43.4 ± 2.3 in group SeIV and 43.0 ± 1.3 in group SeVI was not significantly different at the beginning of the experiment. At a constant and similar feed intake over the whole experiment of 7.53 ± 0.24 g (Fig. 1a) the animals of groups 0Se and SeIV reached slightly different final body weights of 52.3 ± 3.1 and 49.6 ± 4.9, respectively (Fig. 1b). Especially in the second half of the experiment the feed-efficiency ratio in the selenite-treated mice was lower in tendency compared with selenium-deficient mice. Selenate-treated mice had a significantly lower average feed consumption during the whole experiment. In the first 2 weeks of the trial feed consumption in selenate-treated animals (6.81 ± 0.13 g/day) was lower in tendency ($p \leq 0.1$) in comparison with groups 0Se (7.56 ± 0.19) and SeIV (7.47 ± 0.47). From the third week onwards feed consumption in selenate-treated mice was significantly lower ($p < 0.01$) in comparison with their selenium-deficient and selenite-treated companions and fell further to a level of 4.75 ± 0.08 g from sixth week onwards. In accordance with the lower feed consumption, the final body weight of these animals (46.3 ± 2.7 g) was significantly lower ($p < 0.01$) compared with selenium-deficient animals (52.3 ± 3.1). The final body weight of selenite-treated animals lay in a range between the body weight of selenium-deficient mice and selenate-treated mice and did not differ significantly.

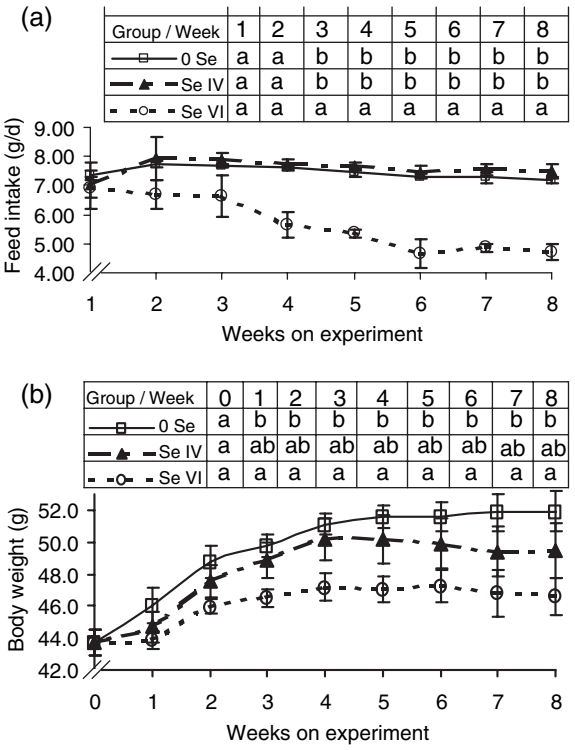


Fig. 1 Development of feed consumption (mean ± SD) (a) and body weight (Mean ± SE) (b) of dbdb mice treated with selenate for 8 weeks (SeVI) in comparison with selenium-deficient (OSe) and selenite-treated mice (SeIV). [significant differences (*P*-minimum < 0.05) between mean values in a column (Tukey test) are indicated by different letters] $n = 7$ animals per group.

Development of selenium status as indicated by plasma glutathione peroxidase and cellular glutathione peroxidase in the liver

Plasma glutathione peroxidase is a sensitive and rapidly determinable indicator of whole body selenium status as the enzyme is predominantly synthesized in the kidney and subsequently released into plasma. The initial GPx3 activity (4.74 ± 1.43 U/ml), measured before subjecting the mice to specified dietary conditions indicated a relatively low selenium status of the mice at the beginning of the study, which was further diminished by 8 weeks of selenium deficiency (group 0Se) in tendency ($p \leq 0.1$) and significantly ($p < 0.01$) improved in mice treated with selenite (SeIV) or selenate (SeVI) for 8 weeks (Table 1). GPx1 activity in the liver, as a parameter for the final selenium status, also reflected the different dietary conditions. Mice kept on selenium-deficient diet for 8 weeks showed a significantly lower GPx1 activity compared with the selenite and selenate-treated controls ($p < 0.01$).

Table 1 GPx1 activity (U/mg protein, mean ± SD) in the liver of dbdb mice kept on selenium deficiency for 8 weeks (0Se) or treated with selenite (SeIV) or selenate (SeVI) for 8 weeks and GPx3 activity (U/ml) in plasma at beginning and end of experiment (n = 7 animals per group)

	Group			
Organ	Initial (0 weeks)	0Se (8 weeks)	SeIV (8 weeks)	SeVI (8 weeks)
GPx1				
Liver	–	171 ± 25.9[a]	703 ± 150[c]	369 ± 43.1[b]
GPx3				
Plasma	4.74 ± 1.43[a]	3.59 ± 1.18[a]	11.86 ± 2.46[b]	8.67 ± 3.27[b]

Significant differences ($p < 0.01$, Tukey test) between groups are indicated by different superscripts within a row.

Development of insulin sensitivity

Fig. 2 compares the effect of 8 weeks of the varying dietary conditions on insulin sensitivity in dbdb mice. The fasting blood glucose concentration prior to IST was significantly ($p < 0.01$) lower in selenate-treated mice (SeVI: 9.19 ± 1.89) in comparison with selenium-deficient (0Se: 19.5 ± 1.35) and selenite-treated mice (SeIV: 25.8 ± 1.41) as well as in comparison with their initial status (13.9 ± 1.56). In selenate-treated mice (SeVI), the development of blood glucose concentration after the insulin challenge was very similar to the data collected 8 weeks earlier for the initial status, indicating that the insulin sensitivity in dbdb mice was kept high by selenate treatment. The most distinct reduction of blood glucose concentration in relation to the respective fasting value was observed 120 min after the insulin challenge in mice of the initial status and in selenate-treated mice (initial: 79.6% reduction of the respective fasting value; SeVI: 80.2%). In selenite-treated and selenium-deficient mice 120 min after the insulin challenge, the reduction of the respective fasting blood glucose value was only 40.1% and 72.8% respectively. Furthermore insulin properties were most sustained in selenate-treated mice (180 min: 72.0% reduction of the respective fasting blood glucose value, 240 min: 48.8% and 300 min: 51.5%) and in the initial status (180 min: 68.8%, 240 min: 49.9% and 300 min: 60.9%). In selenium-deficient mice the return of blood glucose concentration to the fasting value was the most rapid (180 min: 26.3% reduction of the respective fasting value, 240 min: 8.7% and 300 min: even exceeding the fasting value by 12.7%), indicating a significantly impaired insulin sensitivity. In comparison with selenate treatment and with the initial status 8 weeks of selenite treatment also led to a significant reduction of insulin properties (180 min: 56.4% reduction of the respective fasting blood glucose value, 240 min: 40.6% and 300 min: 24.7%).

Activity of PTPs in liver and skeletal muscle

The activity of PTPs in the liver cytosol of selenate-treated dbdb mice (group SeVI) was reduced significantly ($p < 0.01$) by 34.7% and 44.5% in comparison with the activities of selenium-deficient mice (group 0Se) and mice with selenite treatment

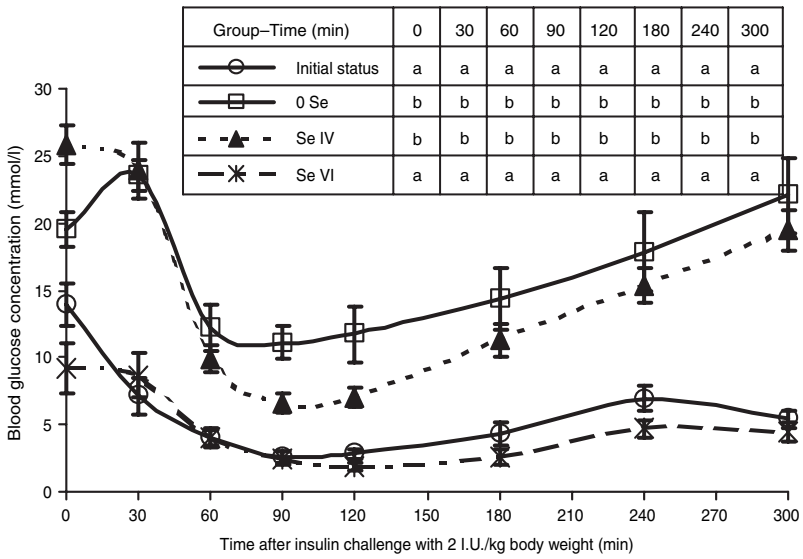


Fig. 2 Reaction of fasting blood glucose concentration (M ± SEM) after an insulin challenge with 2 IU insulin/kg body weight in dbdb mice after 8 weeks of selenate treatment in comparison with selenium-deficient and selenite-treated mice and to their initial status. Significant differences (*P*-minimum < 0.05) between mean values in a column (Dunnett T3 test) are indicated by different letters ($n = 7$ animals per group).

(group SeIV) (Fig. 3). The overall physiological PTP activity in the cytosol of skeletal muscle was about half of the activity measured in the liver cytosol. Eight weeks of selenate treatment significantly ($p < 0.05$) reduced PTP activity to 77.6% and 75.1% of that measured in selenium-deficient and selenite-treated mice, respectively indicating that the ameliorated insulin sensitivity in selenate-treated dbdb mice presumably was related to the reduction (inhibition) of PTP activity.

In vitro inhibitory effect of different selenium compounds on the activity of PTPs

To confirm the results from the *in vivo* trial, an *in vitro* inhibition assay carried out with dbdb mouse liver cytosol was developed to study the inhibition of PTPs by different selenium metabolites (Fig. 4a–e). The addition of selenate (oxidation state +VI) to the reaction mixture, reaching final selenium concentrations of 25, 50, 100, 250, 500, 1000, 2500 and 5000 μmol/l left PTP activity unchanged (Fig. 4a). Adding selenium compounds of the oxidation state +IV, either obtained from the non-enzymatic reduction of selenate with 37% HCl or by the addition of pure selenite or selenious acid to the reaction mixture strongly inhibited the activity of PTPs in a concentration-dependent manner (Fig. 4b–d). A 50% inhibition of PTP activity was obtained with all the above-mentioned selenium compounds of the oxidation state +IV when their final concentration in the assay mixture ranged between 500 and 1000 μmol/l. The inhibition of PTPs by tetravalent selenium compounds steadily increased up to the highest concentration examined in the test (5000 μmol/l Se), reaching a 65% inhibition by non-enzymatically reduced selenate and an 85% and 76% inhibition by the addition of pure selenite and selenious acid respectively. Selenotrisulphides (oxidation state 0), synthesized from selenite and reduced glutathione effected a significantly lower inhibition of PTPs in comparison with the tetravalent selenium compounds (Fig. 4e). 100, 250, 500 and 1000 μmol/l of selenotrisulphides inhibited PTP activity only by 9, 17, 18 and 24%. Even with 5000 μmol/l selenotrisulphides the inhibition of PTPs remained below 50%.

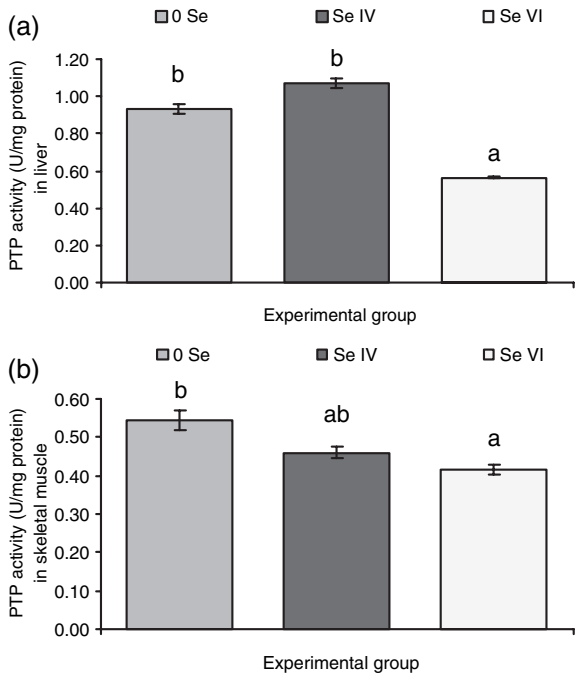


Fig. 3 Effect of 8 weeks of selenate treatment (SeVI) on the activity of protein tyrosine phosphatases (PTPs) (mean ± SD) in the liver (a) and (b) of dbdb mice in comparison with selenium-deficient mice and selenite-treated mice. [Significant differences (liver $p < 0.05$, muscle $p < 0.05$) between mean values (Tukey test) are indicated by different letters] ($n = 7$ animals per group).

Discussion

Feed consumption and development of body weight

In contrast to our observations (Müller et al., 2003) for older male dbdb mice treated with lower selenate doses (15% of the LD_{50} for mice), in the present study selenate treatment led to a marked reduction of feed consumption and a significantly reduced weight gain.

In dbdb mice, the major abnormalities with regard to obesity and diabetes are mediated by deficient expression of the long form of the leptin receptor (LRb). Impaired signalling through the LRb cascade further influences the expression of neuropeptide Y (NPY), responsible for the regulation of fertility, growth and glycaemic control and of α-melanocortin, responsible for the regulation of body weight and energy expenditure (Bates et al., 2003). Studies with tissue cultures showed that the above-mentioned signalling processes can be additionally impaired by the action of protein tyrosine phosphatase 1B (PTP1B). Therefore, it can be speculated that the lower feed consumption and body weight in selenate-treated mice and some of the antidiabetic effects are the consequence of PTP inhibition in the hypothalamus (Kaszubska et al., 2002). To confirm this hypothesis further *in vivo* studies on effects of selenate in the brain and studies in hypothalamic cell lines are necessary.

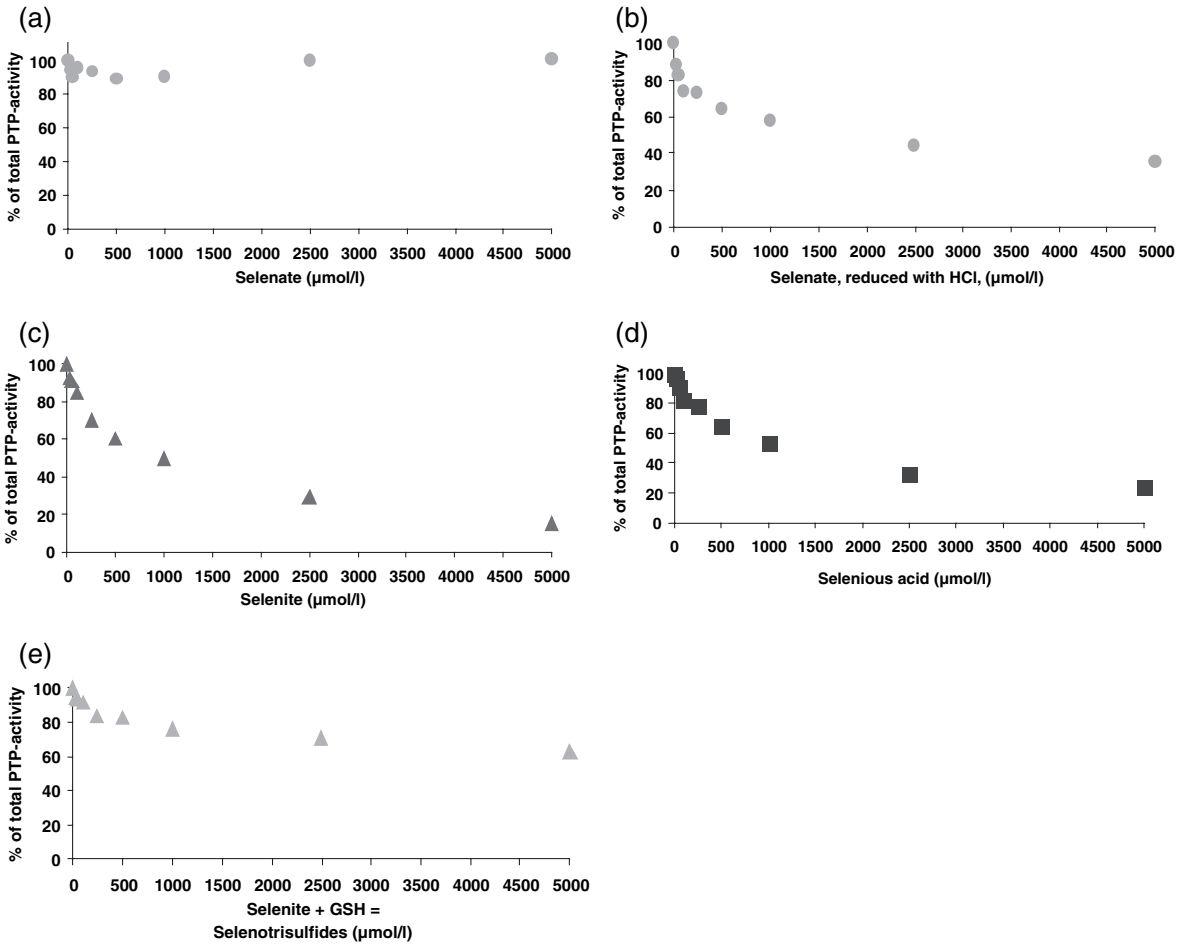


Fig. 4 Results of the *in vitro* inhibition assay to study the inhibitory effect of increasing concentrations of different selenium compounds on the loss of activity of protein tyrosine phosphatases (PTPs). (Each data point represents the mean of three replications).

Selenium status

Plasma glutathione peroxidase is a sensitive and rapidly determinable indicator of whole body selenium status as the enzyme is predominantly synthesized in the kidney and subsequently released into plasma (Cheng et al., 1997; Schwaab et al., 1998; Müller et al., 2002). In the present study, the initial value for GPx3 of 4.74 ± 1.43 U/ml indicated a relatively low selenium status of the mice. Reflecting the low dietary selenium concentration GPx3 declined further in the selenium deficient mice and its activity was markedly improved in mice with selenite and selenate supplementation. Final GPx1 activity in the liver clearly indicated a marked selenium depletion in group 0Se. GPx1 activity in the selenium supplemented groups SeIV and SeVI reflected a high selenium status in these organs despite evidence for a different bioavailability, distribution and excretion profile of selenate and selenite metabolites.

Activity of PTPs in the liver and skeletal muscle and inhibition of PTPs by SeIV compounds

In the current study, a significant decrease of PTP activity in the liver and skeletal muscle could be obtained by oral selenate administration. Within PTPs a 230 amino acid domain, which includes the active centre of the enzymes, is a highly conserved region in the protein structure. A cysteine residue in this region is involved in the hydrolysis of protein phosphotyrosine residues by the formation of a cysteinyl–phosphate intermediate (Denu and Dixon, 1998; Barrett et al., 1999; Salmeen et al., 2003; Van Montfort et al., 2003). In recent years of particular interest is PTP1B, which is involved in the negative regulation of insulin signalling. At present, the expression and activity of PTP1B in rodents and other mammalian species is controversially discussed (Harley and Levens, 2003; Ramachandran and Kennedy, 2003; Tonks, 2003). However, there is no

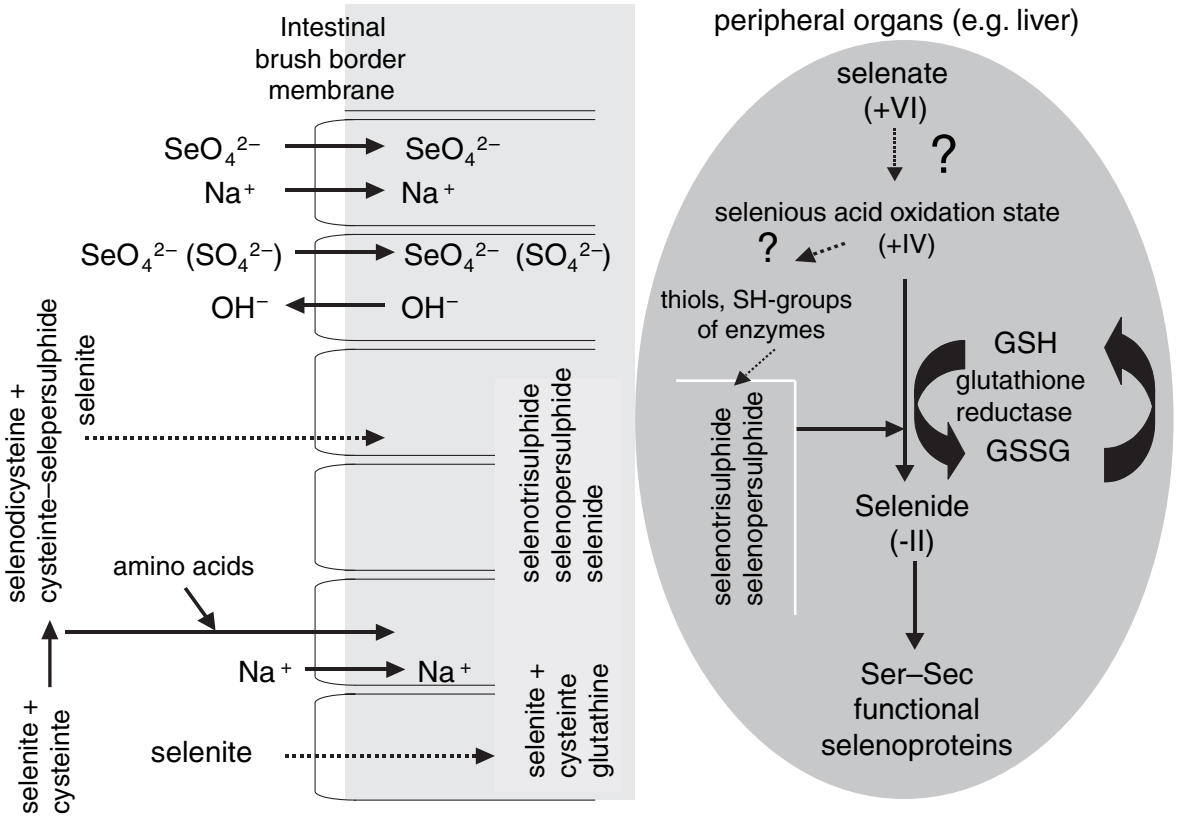


Fig. 5 Selenium absorption and metabolism according to Wolffram *et al.* (1985) and Behne *et al.* (1991).

doubt that diabetic symptoms can be efficiently reduced by treatment with PTP1B enzyme inhibitors or antisense oligonucleotides, which reduce the mRNA expression and the protein synthesis of the enzyme (Zinker et al., 2002; Asante-Appiah and Kennedy, 2003; Gum et al., 2003; Lam et al., 2004). An explanation of a mechanism for the reversible and irreversible PTP1B inhibition has been recently suggested and is based on the stepwise oxidation of the active site cysteine SH group (Van Montfort et al. 2003; Denu and Dixon, 1998; Barrett et al., 1999).

In the present study, the *in vitro* inhibition assay was performed to investigate whether the observed PTP reduction by selenate *in vivo* is based on the inhibition of these enzymes. Initially surprising and contrary to the *in vivo* results, the incubation of dbdb mouse liver cytosol with increasing selenate concentrations (Se oxidation state +VI) effected no inhibition of PTPs, whereas the incubation with SeIV compounds either obtained by non-enzymatic reduction of selenate or by the addition of pure SeIV-derivatives (selenite and selenious acid) led to a concentration-dependent inhibition of PTP activity. A possible explanation for this observation is that free SeIV compounds act as the actual inhibitors of PTPs. These compounds can derive from the different metabolism of selenate and selenite (Fig. 5). In mammals, selenate is absorbed unmodified by a sodium-dependent co-transport-system, which is also involved in sulphate absorption and by an anion exchange mechanism. Further, there is evidence that selenate is distributed unmodified to peripheral tissues, where it is reduced stepwise to oxidation state −II. Details of the final selenate metabolism in mammalian tissues are not yet clear. Either selenate is metabolized in a similar manner as sulphate or it undergoes reduction during which the oxidation state +IV is formed as an intermediate. As a result of the reduction in PTP activity observed in selenate-treated mice, the last mentioned pathway seems to play an important role.

In contrast to selenate most of the selenite does not reach the peripheral tissues in the oxidation state +IV as selenite forms selenotrisulphides (oxidation state 0) during its intestinal absorption. These selenotrisulphides are distributed to organs on the periphery and undergo reduction to the oxidation state −II, from which they can be utilized for the

synthesis of functional selenoproteins. This is supported by the results from the *in vitro* inhibition test. When selenite and GSH were converted to selenotrisulphides prior to their use in the *in vitro* inhibition test, the inhibition of PTPs is decreased significantly. The remaining inhibition may have derived from not fully completed synthesis of selenotrisulphides in the model investigated. This must be examined in future investigations with purified selenotrisulphides and pure preparations of PTP1B. Further, the examination of the precise inhibition mechanism of SeIV compounds on PTPs in general and on PTP1B in particular seems to be an interesting subject for future investigations using mass spectrometry. From the knowledge of selenium biochemistry, it can be speculated that selenious acid and its derivatives react with the active site cysteine residue of PTPs. Moreover, changes in the cellular status of reduced and oxidized thiols must be the subject of future investigations, as these compounds are known to be involved in the redox regulation and inactivation of PTPS.

Moreover, investigations with type I diabetic rats or tissue cultures would be of interest in order to examine if the insulinomimetic effects of selenate including an increase in phosphorylation of the β-subunit of the insulin receptor and of downstream components are the result of PTP inhibition.

Toxicological aspects of the selenium doses given in the present study

Both the data from the *in vivo* study and from the *in vitro* inhibition assay suggest that the inhibition of PTPs needs supranutritive doses of selenium. The consumption of 5 g feed per day, containing the recommended selenium amount for mice (0.2 mg/kg diet, NRC, 1995), would result in a daily uptake of 1 μg selenium. The LD_{50} of selenate and selenite for mice is 3500 μg/kg body weight (Hall et al., 1951; Wilber, 1980; Olson, 1986). Derived from the molecular weight in the case of selenate selenium accounts for 42% of this amount (1470 μg/kg body weight). In the *in vivo* study, 15–35% of the LD_{50} of selenate was administered to the mice daily. This amounts to an absolute selenium uptake of 10–25 μg (10–25-fold of the recommended dietary amount) at a mean body weight of 50 g.

In the *in vitro* trial, the onset of an effective inhibition of PTPs ranged between 50 and 100 μmol/Lol Se/l (3.95–7.9 μg Se/l), thus it can be assumed that with the oral application of selenate at regular intervals in the doses used in our present study, sufficient selenium concentrations for the inhibition of PTPs can be obtained.

The possibility of chronic selenium toxicity cannot be excluded for the doses applied in the present study. The results from a long-term study on selenium toxicity in rats, however suggest that selenium concentrations up to 4 mg/kg diet (20-fold of the recommended amount) do not affect animal health (Jacobs and Forst, 1981). In this study, the mortality rate after 2 years (survival rate >90%) in the group which received 4 mg selenium/kg diet was not higher than in the group fed with 1 mg selenium/kg diet. In general, information on selenium toxicity is limited. Further investigations into the precise amount of selenium needed for the treatment of diabetes and on the long-term toxicity in different animal species are needed.

Conclusion

The results of the present study suggest that the insulin sensitizing role of selenate *in vivo* is partially based on the inhibition of PTPS by SeIV compounds, which seem to be generated during selenate metabolism. This can be concluded from the results of the *in vitro* inhibition test, where selenate showed no inhibition of PTPs, whereas selenious acid derivatives were found to be the actual inhibitors of PTPs.

References

Asante-Appiah, E.; Kennedy, B. P., 2003: Protein tyrosine phosphatases, the quest for negative regulators of insulin action. *American Journal of Physiology, Endocrinology and Metabolism* **284**, E663–E670.

Barrett, W. C.; DeGnore, J. P.; Koenig, S.; Fales, H. M.; Keng, Y. F.; Zhang, Z. Y.; Yim, M. B.; Chock, P. B., 1999: Regulation of PTP1B via glutathionylation of the active site cysteine 215. *Biochemistry* **28**, 6699–6705.

Bates, S. H.; Stearns, W. H.; Dundon, T. A.; Schubert, M.; Tso, A. W.; Wang, Y.; Banks, A. S.; Lavery, H. J.; Haq, A. K.; Maratos-Flier, E.; Neel, B. G.; Schwartz, M. W.; Ganther, H. E.; Myers, M. G. Jr., 2003: STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* **421**, 856–859.

Battell, M. L.; Delgatty, H. L.; McNeill, J. H., 1998: Sodium selenate corrects glucose tolerance and heart function in STZ diabetic rats. *Molecular and Cellular Biochemistry* **179**, 27–34.

Becker, D. J.; Reul, B.; Ozcelikay, A. T.; Buchet, J. P.; Henquin, J. C.; Brichard, S. M., 1996: Oral selenate improves glucose homeostasis and partly reverses

abnormal expression of liver glycolytic and gluconeogenic enzymes in diabetic rats. *Diabetologia* **39**, 3–11.

Behne, D.; Kyriakopoulos, A.; Scheid, S.; Gessner, H., 1991: Effects of chemical form and dosage on the incorporation of selenium into tissue proteins. *Journal of Nutrition* **121**, 806–814.

Berg, E. A.; Wu, J. Y.; Campbell, L.; Kagey, M.; Stapleton, S. R., 1995: Insulin-like effects of vanadate and selenate on the expression of glucose-6-phosphate dehydrogenase and fatty acid synthase in diabetic rats. *Biochimie* **77**, 919–924.

Bradford, M. M., 1976: A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.

Brigelius-Flohé, R., 1999: Tissue-specific functions of individual glutathione peroxidases. *Free Radical Biology and Medicine* **27**, 951–965.

Cheng, W. H.; Ho, Y. S.; Ross, D. A.; Han, Y.; Combs, G. F., Jr; Lei, X. G., 1997: Overexpression of cellular glutathione peroxidase does not affect expression of plasma glutathione peroxidase or phospholipid hydroperoxide glutathione peroxidase in mice offered diets adequate or deficient in selenium. *Journal of Nutrition* **127**, 675–680.

Denu, J. M.; Dixon, J. E., 1998: Protein tyrosine phosphatases, mechanisms of catalysis and regulation. *Current Opinion in Chemical Biology* **2**, 633–641.

Ganther, H. E., 1968: Selenotrisulphides. Formation by the reaction of thiols with selenious acid. *Biochemistry* **7**, 2898–2905.

Gum, R. J.; Gaede, L. L.; Koterski, S. L.; Heindel, M.; Clampit, J. E.; Zinker, B. A.; Trevilyan, J. M.; Ulrich, R. G.; Jirousek, M. R.; Rondinone, C. M., 2003: Reduction of protein tyrosine phosphatase 1B increases insulin-dependent signaling in ob/ob mice. *Diabetes* **52**, 21–28.

Hall, R. H.; Laskin, S.; Frank, P.; Maynard, E. A.; Hodge, H. C., 1951: Preliminary observations on the toxicity of elemental selenium. *AMA Archives of Industrial Hygiene and Occupational Medicine* **4**, 458–464.

Harley, E. A.; Levens, N., 2003: Protein tyrosine phosphatase 1B inhibitors for the treatment of type 2 diabetes and obesity, recent advances. *Current Opinion in Investigational Drugs* **4**, 1179–1189.

Hei, Y. J.; Farahbakshian, S.; Chen, X.; Battell, M. L.; McNeill, J. H., 1998: Stimulation of MAP kinase and S6 kinase by vanadium and selenium in rat adipocytes. *Molecular and Cellular Biochemistry* **178**, 367–375.

Jacobs, M.; Forst, C., 1981: Toxicological effects of sodium selenite in Sprague–Dawley rats. *Journal of Toxicology and Environmental Health* **8**, 575–585.

Kaszubska, W.; Falls, H. D.; Schaefer, V. G.; Haasch, D.; Frost, L.; Hessler, P.; Kroeger, P. E.; White, D. W.; Jirousek, M. R.; Trevillyan, J. M., 2002: Protein tyrosine phosphatase 1B negatively regulates leptin signaling in a hypothalamic cell line. *Molecular and Cellular Endocrinology* **195**, 109–118.

Koehrle, J., 1996: Thyroid hormone deiodinases – a selenoenzyme family acting as gate keepers to thyroid hormone action. *Acta Medica Austriaca* **23**, 17–30.

Lam, N. T.; Lewis, J. T.; Cheung, A. T.; Luk, C. T.; Tse, J.; Wang, J.; Bryer-Ash, M.; Kolls, J. K.; Kieffer, T. J., 2004: Leptin increases hepatic insulin sensitivity and protein tyrosine phosphatase 1B expression. *Molecular Endocrinology* **18**, 1333–1345.

May, J. M.; Mendiratta, S.; Hill, K. E.; Burk, R. F., 1997: Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. *Journal of Biology and Chemistry* **272**, 22607–22610.

McNeill, J. H.; Delgatty, H. L.; Battell, M. L., 1991: Insulin-like effects of sodium selenate in streptozotocin-induced diabetic rats. *Diabetes* **40**, 1675–1678.

Most, E.; Pallauf, J., 1999: *Bestimmung von Selen in biologischen Matrices mittels Hydrid- and Graphitrohr-AAS*. VDLUFA-Kongress, Halle, Saale, Book of Abstracts, 158.

Müller, A. S.; Pallauf, J.; Most, E., 2002: Parameters of dietary selenium and vitamin E deficiency in growing rabbits. *Journal of Trace Elements in Medicine and Biology* **16**, 47–55.

Müller, A. S.; Pallauf, J.; Rafael, J., 2003: The chemical form of selenium affects insulinomimetic properties of the trace element, investigations in type II diabetic dbdb mice. *Journal of Nutrition and Biochemistry* **14**, 637–647.

National Research Council (NRC), 1995: *Nutrient Requirements of Laboratory Animals*, 4th revised edn. National Academy Press, Washington, DC.

Olson, O. E., 1986: Selenium toxicity in animals with emphasis on man. *Journal of American College of Toxicology* **5**, 4570.

Ramachandran, C.; Kennedy, B. P., 2003: Protein tyrosine phosphatase 1B, a novel target for type 2 diabetes and obesity. *Current Topics in Medical Chemistry* **3**, 749–757.

Salmeen, A.; Andersen, J. N.; Myers, M. P.; Meng, T. Z.; Hinks, J. A.; Tonks, N. K.; Barford, D., 2003: Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature* **423**, 769–773.

Schwaab, V.; Faure, J.; Dufaure, J. P.; Drevet, J. R., 1998: GPx3, the plasma-type glutathione peroxidase is expressed under androgenic control in the mouse epididymis and vas deferens. *Molecular Reproduction and Development* **51**, 362–372.

Self, W. T.; Tsain, L.; Stadtman, T. C., 2000: Synthesis and characterization of selenotrisulphide derivatives of lipoic acid and lipoamide. *Proceedings of the National Academy of Sciences USA* **97**, 12481–12486.

Shiobara, Y.; Ogra, Y.; Suzuki, K. T., 1999: Speciation of metabolites of selenate in rats by HPLC-ICP-MS. *Analyst* **124**, 1237–1241.

Stapleton, S. R., 2000: Selenium, an insulin-mimetic. *Cellular and Molecular Life Science* **57**, 1874–1879.

Stapleton, S. R.; Garlock, G. L.; Foellmi-Adams, L.; Kletzien, R. E., 1997: Selenium potent stimulator of tyrosyl phosphorylation and activator of MAP kinase. *Biochimica et Biophysica Acta* **1355**, 259–326.

Sunde, R. A.; Evenson, J. K., 1987: Serine incorporation into the selenocysteine moiety of glutathione peroxidase. *Journal of Biology and Chemistry* **262**, 933–937.

Suzuki, K. T.; Ogra, Y., 2002: Metabolic pathway for selenium in the body, speciation HPLC-ICP MS with enriched Se. *Food Additives and Contaminants* **19**, 974–983.

Tappel, M. E.; Chaudiere, J.; Tappel, A. L., 1982: Glutathione peroxidase activities of animal tissues. *Comparative Biochemistry and Physiology* **73**, 945–949.

Tonks, N. K., 2003: PTP1B, from the sidelines to the front lines! *FEBS Letters* **546**, 140–148.

Van Montfort, R. L.; Congreve, M.; Tisi, D.; Carr, R.; Jhoti, H., 2003: Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. *Nature* **423**, 773–777.

Walczak, R.; Hubert, N.; Carbon, P.; Krol, A., 1997: Solution structure of SECIS, the mRNA element required for eukaryotic selenocysteine insertion – interaction studies with the SECIS-binding protein SBP. *Biomedical and Environmental Science* **10**, 177–181.

Wilber, C. G., 1980: Toxicology of selenium: a review. *Clinical Toxicology* **17**, 171–230.

Wolffram, S.; Ardueser, F.; Scharrer, F., 1985: In vivo intestinal absorption of selenate and selenite by rats. *Journal of Nutrition* **115**, 454–459.

Wolffram, S.; Berger, B.; Grenacher, B.; Scharrer, E., 1989: Transport of selenoamino acids and their sulphur analogues across the intestinal brush border membrane. *Journal of Nutrition* **119**, 706–712.

Zhu, L.; Goldstein, B., 2002: Use of an anaerobic chamber environment for the assay of endogenous cellular protein tyrosine phosphatase activities. *Biological Proceedings Online* **4**, 1–9.

Zinker, B. A.; Rondinone, C. M.; Trevillyan, J. M.; Gum, R. J.; Clampit, J. E.; Waring, J. F.; Xie, N.; Wilcox, D.; Jacobson, P.; Frost, I.; Kroeger, P. E.; Reilly, R. M.; Koterski, S.; Opgenorth, T. J.; Ulrich, R. G.; Crosby, S.; Butler, M.; Murray, S. F.; McKay, R.; Bhanot, S.; Monia, B. P.; Jirousek, M. R., 2002: PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. *Proceedings of the National Academy of Sciences of USA* **99**, 11357–11362.

Available online at www.sciencedirect.com

SCIENCE DIRECT

Journal of Nutritional Biochemistry 17 (2006) 548–560

Journal of Nutritional Biochemistry

Compendium of the antidiabetic effects of supranutritional selenate doses. In vivo and in vitro investigations with type II diabetic *db/db* mice

Andreas S. Mueller, Josef Pallauf*

Institute of Animal Nutrition and Nutritional Physiology, Justus Liebig University Giessen, D-35392 Giessen, Germany

Received 23 August 2005; received in revised form 14 October 2005; accepted 15 October 2005

Abstract

In recent years, a number of investigations on the antidiabetic effects of supranutritional selenate doses have been carried out. Selenate (selenium oxidation state +VI) was shown to possess regulatory effects on glycolysis, gluconeogenesis and fatty acid metabolism, metabolic pathways which are disturbed in diabetic disorders. An enhanced phosphorylation of single components of the insulin signalling pathway could be shown to be one molecular mechanism responsible for the insulinomimetic properties of selenate. In type II diabetic animals, a reduction of insulin resistance could be shown as an outcome of selenate treatment. The present study with *db/db* mice was performed to investigate the antidiabetic mechanisms of selenate in type II diabetic animals.

Twenty-one young adult female *db/db* mice were randomly assigned to three experimental groups (selenium deficient=0Se, selenite-treated group=SeIV and selenate-treated group=SeVI) with seven animals each. Mice of all groups were fed a selenium-deficient diet for 8 weeks. The animals of the groups SeIV and SeVI were supplemented with increasing amounts of sodium selenite or sodium selenate up to 35% of the LD_{50} in week 8 in addition to the diet by tube feeding.

Selenate treatment reduced insulin resistance significantly and reduced the activity of liver cytosolic protein tyrosine phosphatases (PTPs) as negative regulators of insulin signalling by about 50%. In an in vitro inhibition test selenate (oxidation state +VI) per se did not inhibit PTP activity. In this test, however, selenium compounds of the oxidation state +IV were found to be the actual inhibitors of PTP activity.

Selenate administration in vivo further led to characteristic changes in the selenium-dependent redox system, which could be mimicked in an in vitro assay and provided further evidence for the intermediary formation of SeIV metabolites. The expression of peroxisome proliferator-activated receptor gamma (PPARγ), another important factor in the context of insulin resistance and lipid metabolism, was significantly increased by selenate application. In particular, liver gluconeogenesis and lipid metabolism were influenced strongly by selenate treatment.

In conclusion, our results showed that supranutritional selenate doses influenced two important mechanisms involved in insulin-resistant diabetes, namely, PTPs and PPARγ, which, in turn, can be assumed as being responsible for the changes in intermediary metabolism, e.g., gluconeogenesis and lipid metabolism. The initiation of these mechanisms thereby seems to be coupled to the intermediary formation of the selenium oxidation state +IV (selenite state) from selenate.

Keywords: Antidiabetic effects; Selenate; Protein tyrosine phosphatase

1. Introduction

When taken up at the recommended level (animals: 0.15–0.30 mg Se/kg dietary dry matter; humans: 50–70 μg Se daily), selenium performs its physiological functions in the body of animals and humans as an integral part of the redox-active centre of functional selenoproteins [1–5]. The detoxification of peroxides, the involvement in the regulation of thyroid hormone metabolism and the participation in the reduction of disulfides and ascorbate are the most important functions fulfilled by the functional selenoproteins, glutathione peroxidase, iodothyronine deiodinase and thioredoxin reductase [6–8].

In human food, selenium is present in two major forms. Feedstuffs derived from animal sources mainly contain selenium in the form of selenocysteine from functional selenoproteins, whereas selenium from plant-derived foodstuffs is present predominantly as selenomethionine. In

* Corresponding author. Tel.: +49 641 9939231; fax: +49 641 9939239.
E-mail address: josef.pallauf@ernaehrung.uni-giessen.de (J. Pallauf).

0955-2863/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.10.006

trace element supplements, selenium is frequently added in the form of inorganic salt, sodium selenite (selenium oxidation state +IV and sodium selenate +VI). Selenium from various dietary sources is absorbed in the jejunum and in the ileum of mammals. The amino acid derivatives selenomethionine and selenocysteine use the same carriers as their sulphur analogues methionine and cysteine [9]. Selenate uses a sodium-sulphate cotransporter for its absorption, which is driven by the activity of Na^+/K^+-ATPase at the basolateral enterocyte membrane [10]. In contrast, selenite prior to its absorption partially reacts with glutathione and other thiols in the lumen to form selenotrisulfides, which are presumably taken up into the enterocytes by amino acid transporters. Another part of selenite diffuses through the apical membrane and reacts with thiols in the cytosol of enterocytes [10]. The selenium compounds mentioned above are absorbed, to a high extent (> 85%), from dietary sources, but differences exist in the absorption time. As a result of the upstream selenotrisulfide synthesis, selenite absorption is slower than selenate and selenomethionine absorption [10]. Subsequently, the selenocompounds are released into the blood stream at the basolateral enterocyte membrane and distributed to the various peripheral tissues. The exact transport mechanism for the various selenium compounds is not fully understood yet. Selenomethionine associates with hemoglobin, while selenate and the remaining free selenite were found to be transported with α- and γ-globulins [11,12]. Thus, orally administered selenite presumably enters the peripheral organs in the form of selenotrisulfides, or it is reduced in the erythrocytes. Selenate is metabolised during and after its unmodified uptake by the peripheral tissues (Fig. 1).

This hypothesis of a distinctly different cellular metabolism for selenite and selenate is supported by an investigation into intermediary selenium metabolites after intravenous injection of rats with both compounds [13,14]. Selenite was rapidly taken up by red blood cells, reduced in the erythrocytes to the selenide oxidation state −II and delivered to peripheral organs (liver) in an albumin-bound form. In contrast, unmodified selenate could be detected in the bloodstream, and the successive reduction to the oxidation state −II takes place during selenate uptake from plasma to peripheral organs. A fraction of "acid labile selenium" consisting of selenium bound unspecifically to proteins (presumably via the formation of Se-S bonds) could be detected. After intravenous injection with both compounds, the main excretion products detected in urine consisted of the methylated forms of selenium (monomethylselenol and trimethylselenonium ion). Injection of selenite (SeIV) led to a major peak of these methylated metabolites in urine after 0–6 h in comparison to a selenate (SeVI) injection, which showed high metabolite concentrations after 6–12 h. Additionally, unmodified selenate was excreted after selenate injection [14].

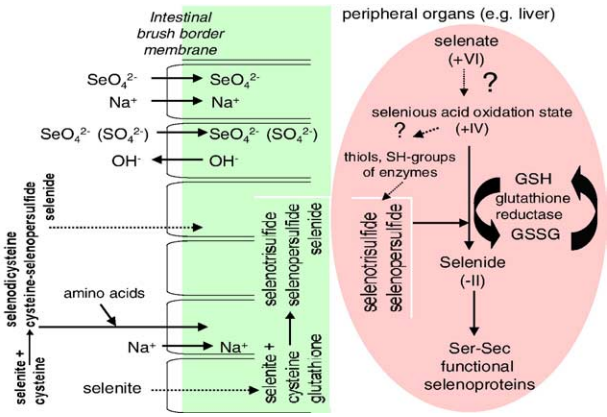


Fig. 1. Current comprehension of mammalian selenium absorption and metabolism.

Selenomethionine is the only selenium compound that can be incorporated unspecifically into proteins instead of its sulphur analogue methionine. The ongoing cellular metabolism of all selenium compounds requires a step-by-step glutathione-dependent reduction to the selenide oxidation state −II, which is the physiological basis for the incorporation of the trace element into the selenocysteine residue of functional selenoproteins by a cotranslational mechanism [15–17].

In recent years, a fascinating new physiological aspect has been found for selenate. Selenate administration in supranutritive doses (daily administration of amounts up to the individual LD_{50} for about 8 weeks) to rats with streptocotozin-induced type I diabetes led to a sustained correction of their diabetic status including the decrease of the elevated blood glucose concentration and considerable changes in the expression of abnormally expressed glycolytic and gluconeogenic marker enzymes [18–24]. From in vivo experiments and in vitro studies with tissue cultures, it was concluded that enhanced phosphorylation reactions at the β subunit of the insulin receptor and further components of the insulin signalling cascade are responsible for the so-called insulinomimetic properties of selenate [25,26].

Oral treatment of mice with alloxan-induced type I diabetes with a high dose of selenite (4 mg/kg body weight per day) failed to reduce hyperglycemia in these animals, which seems to be based on differences in the intermediary metabolism of selenite and selenate [27].

Insulinomimetic properties of selenate could also be found in type II diabetic *db/db* mice. In this animal model featuring severe symptoms of type II diabetes [28,29], the antidiabetic effect of selenate could be attributed to the reduction of insulin resistance, whereas the in vivo administration of selenite did not result in a significant amelioration of insulin resistance and diabetes [28].

Besides the influence of insulin and therefore of insulin sensitising agents on glucose metabolism, hormones also play a crucial role in fatty acid metabolism.

Peroxisome proliferator-activated receptors (PPARs) are originally transcription factors belonging to the superfamily of nuclear receptors, discussed as acting as master regulators of fatty acid metabolism and displaying an important link between fatty acid metabolism and insulin sensitivity. Three isoforms (α, β and γ) have been described. They act on DNA response elements as heterodimers with the nuclear retinoic acid receptor. Their natural activating ligands are fatty acids and lipid-derived substrates. PPARα is present predominantly in the liver and heart and, to a lesser extent, in skeletal muscle. When activated, it promotes fatty acid oxidation, ketone body synthesis and glucose sparing. Peroxisome proliferator-activated receptor gamma (PPARγ) is considered to be one of the master regulators of adipocyte differentiation. The isoform PPARγ2 is abundantly expressed in mature adipocytes and is elevated in animals with fatty livers.

Thiazolidinediones were developed as antidiabetic drugs acting as synthetic ligands of PPARs. They increase peripheral glucose utilisation and reduce insulin resistance [30,31]. The whole complex of tissue-specific actions and interactions of PPARs is not yet fully understood. In a study with transgenic mice, animals without liver PPARγ but with adipose tissue developed fat intolerance, increased adiposity, hyperlipidemia and insulin resistance. Thus, it was concluded that liver PPARγ regulates triglyceride homeostasis, contributing to hepatic steatosis, but protecting other tissues from triglyceride accumulation and insulin resistance [32,33]. Moreover, it was shown that the treatment of *db/db* mice with thiazolidinediones induced expression in the liver of adipose tissue PPARγ target genes, such as adipocyte FABP [34], which foretells that hepatic lipid accumulation (steatosis) could occur during long-term administration [35,36].

The present study with young female *db/db* mice was therefore carried out to investigate the mechanisms by which selenate influences insulin resistance and metabolic pathways in type II diabetic mice.

2. Materials and methods

2.1. Animals and diets

Twenty-one young female *db/db* mice (C57BL/KsOlaHsd-Leprdb) aged 6 weeks with an average body weight of 43.7±2.03 g were obtained from Harlan/Winkelmann (Borchen, Germany). The animals had previously been fed a standard chow for mice containing 0.25 mg selenium as sodium selenite per kilogram diet. The mice were randomly assigned to three groups of seven animals each (selenium deficient=0Se, selenite-treated group=SeIV and selenate-treated group=SeVI) and individually housed in plastic cages with shavings as bedding material at 22°C room temperature and a 12:12-h light/dark cycle. The animals of all groups were fed a selenium-deficient experimental diet (<0.02 mg Se/kg diet) based on torula yeast (Table 1).

With the exception of Se, the diet was formulated to meet the current recommendations for mice [1]. Mice of the group 0Se were kept on a selenium-deficient diet for 8 weeks. The animals of groups SeIV and SeVI were supplemented with amounts increasing from 15% up to 35% of the LD_{50} of sodium selenite and sodium selenate by week 8 in addition to the diet by tube feeding (LD_{50} of sodium selenite and sodium selenate ~3.5 mg/kg body weight).

After 2 days of recovery from the final insulin resistance test (IRT), the mice of all experimental groups were anaesthetised in a carbon dioxide atmosphere and subsequently decapitated. Organs were immediately removed, frozen in liquid nitrogen and stored at −80°C until analysis. Small pieces from all organs were placed in RNA later and frozen at −20°C for RNA extraction.

The protocol of the animal experiment was approved by the regional council of Giessen.

2.2. Performance of a whole-body insulin sensitivity test

Before subjecting the mice to the specified dietary conditions (initial status) and after 8 weeks under experimental conditions, their whole-body insulin resistance was evaluated.

Insulin sensitivity tests (ISTs) in mice fasted overnight were performed by subcutaneous injection of 2 IU insulin/kg body weight (Insuman Infusat 100 IU/ml from AVENTIS Pharma Deutschland, Frankfurt/Main, Germany). Glucose concentration in blood sampled from

Table 1
Composition of the selenium-deficient basal diet, based on torula yeast

Dietary components	Content (g/kg diet)
Torula yeast	300.0
Cellulose BWW 40	50.0
Soybean oil	25.0
Coconut oil	25.0
DL-Methionine	3.0
Premix of minerals and trace elements (without selenium)[a]	66.6
Premix of vitamins[b]	10.0
Choline chloride	2.0
Wheat (low in selenium)	450.0
Maize starch	68.4
Total	1000

[a] Minerals and trace elements added per kg diet: $CaCO_3$: 12.5 g=5.090 mg Ca/kg diet; KH_2PO_4: 15.0 g=2.650 mg P/kg diet; Na_2HPO_4: 7.5 g=1.630 mg P/kg diet; $MgSO_4 \times 7\ H_2O$: 5.0 g=508 mg Mg/kg diet; NaCl: 4.0 g=1.56 g Na/kg diet; $CuSO_4 \times 5\ H_2O$: 20 mg=5.10 mg Cu/kg diet; $FeSO_4 \times 7\ H_2O$: 250 mg=50.2 mg Fe/kg diet; $ZnSO_4 \times 7\ H_2O$: 150 mg=34.1 mg Zn/kg diet; $MnSO_4 \times H_2O$: 130 mg=47.4 mg Mn/kg diet; $CrCl_3$: 7.5 mg=2.47 mg Cr/kg diet; NaF: 2.2 mg=0.99 mg F/kg diet; KJ: 0.3 mg=0.23 mg J/kg diet; $CoSO_4 \times 7\ H_2O$: 1.2 mg=0.25 mg Co/kg diet; $Na_2MoO_4 \times 2\ H_2O$: 0.5 mg=0.20 mg Mo/kg diet.

[b] Vitamins added per kilogram diet: vitamin A: 15000 IU; vitamin D: 1500 IU; vitamin E: 15 IU; vitamin K_3: 5 mg; vitamin B_1: 10 mg; vitamin B_2: 10 mg; vitamin B_6: 10 mg; vitamin B_{12}: 0.02 mg; niacin: 50 mg; pantothenic acid: 10 mg; biotin: 0.3 mg; vitamin C: 150 mg.

the tail vein was recorded before starting the test and 30, 60, 90, 120, 180, 240 and 300 min after insulin injection. Blood glucose concentration during IRT was determined using a glucometer (Bayer Elite).

The protocol of the animal experiment was approved by the regional council of Giessen.

2.3. Determination of biochemical and physiological parameters

2.3.1. Measurement of parameters of the selenium-dependent redox system in the liver

2.3.1.1. Glutathione peroxidase 1 and test of the influence of different selenium compounds on GPx1 activity in vitro. Glutathione peroxidase 1 was measured in the cytosol of 1:10 (w/v) liver homogenates by the indirect spectrophotometric procedure coupled to glutathione reductase [37]. NADPH oxidation was recorded for 3 min at 340 nm. A blank without added liver cytosol was carried out for each sample. The activity of GPx1 was calculated from the absorption difference. One unit of GPx1 was defined as 1 μmol NADPH oxidized per minute under the described conditions. The activity of GPx1 was normalized to 1 mg protein.

To test the influence of the inorganic selenium compounds selenate (oxidation state +VI) and selenite (oxidation state +IV) on the glutathione peroxidase and glutathione reductase–redox system, final selenium concentrations of up to 1000 μmol/L as selenate and selenite were added to the glutathione peroxidase assay (which contains all components of the glutathione peroxidase redox system: reduced glutathione, glutathione reductase, NADPH and glutathione peroxidase from the sample) as described above, replacing 10 μl of the assay buffer by selenate and selenite dissolved in bidistilled water.

2.3.1.2. Glutathione reductase. The activity of glutathione reductase in the liver of the *db/db* mice was determined using a standard procedure that is coupled to NADPH oxidation [38].

2.3.1.3. Total glutathione and oxidized glutathione. The concentration of total glutathione and oxidized glutathione was analysed according to the standard protocol coupled to glutathione reductase and DTNB [39]. Sample concentrations were calculated from a standard curve prepared with pure glutathione disulfide (GSSG) (concentration range: 0–0.066 μmol GSSG/ml).

2.3.1.4. Thioredoxin reductase. The activity of thioredoxin reductase was determined by the NADPH and DTNB-coupled procedure [40]. Prior to the measurement of thioredoxin reductase activity, the 1:10 (w/v) liver homogenates were dialysed against PBS in order to remove the interfering glutathione. DTNB reduction was measured for 3 min at 412 nm. One unit of thioredoxin reductase activity was defined as 1 μmol DTNB reduced per minute. Enzyme activity was normalised to 1 mg protein.

2.3.2. Determination of the activity of cytosolic protein tyrosine phosphatases (PTPs) in the liver and assay of the "*in vitro inhibition*" of PTPs by different selenium compounds

Protein tyrosine phosphatase activity was determined with modifications according to a method based on the hydrolysis of paranitrophenyl phosphate (pNPP) [41,42] as published earlier. The inhibition of PTP activity by different selenium compounds was developed using pooled liver cytosol from three adult female *db/db* mice fed a standard chow containing 0.25 mg Se/kg diet.

The activity of PTPs was assessed as described above for liver homogenates obtained from the in vivo trial. In addition to the above procedure, 10 μl of aqueous solutions of sodium selenate (oxidation state: +VI), nonenzymatically reduced selenate (using 37% HCl as the reducing agent; oxidation state: +IV), sodium selenite (oxidation state: +IV), selenious acid (oxidation state: +IV) and freshly synthesized selenotrisulfides from the reaction of reduced glutathione and selenite in a molar ratio of 4:1 (synthesized according to a standard protocol [43]; oxidation state: +II), reaching final selenium concentrations of 25–5000 μmol/L, were added before incubation of the reaction mixtures with pNPP for 10 min. A blank without cytosol was carried out for all determinations. The inhibition of PTPs was expressed as the percent inhibition in comparison with the PTP activity obtained in liver cytosol without addition of selenium compounds.

2.3.3. RT-PCR analysis to examine the expression of protein tyrosine phosphatase 1B (PTP1B), PPARγ, fructose-1,6-diphosphatase (F-1,6-Dptase) and phosphoenolpyruvate carboxykinase (PEPCK)

For the RT-PCR analysis of PTP1B expression in the liver, total RNA was prepared using the acid guanidinium thiocyanate extraction method as described previously [44]. The extracted RNA was dissolved in DEPC-treated water, and the concentration and purity were determined in an UV visible photometer at 260 and 280 nm. To check the quality of the RNA preparations, 10 μg of total RNA from each preparation was separated electrophoretically in 1.5% formaldehyde containing agarose gels. The RNA solutions were diluted with DEPC-treated water to a final concentration of 2 μg/μl. From the diluted RNA solutions for each experimental group (0Se, SeIV and SeVI), three RNA pools from two animals were prepared. Five micrograms (2.5 μl) of the RNA pools was used for reverse transcription with a cDNA synthesis kit (RevertAID H Minus First Strand cDNA Synthesis Kit, #K1631 from MBI Fermentas). For this purpose, the procedure using the oligo (dT_{18}) primers was chosen. The cDNA was diluted 1:3 with DEPC-treated water. The use of 2 μl of this diluted cDNA for the amplification of gene-specific fragments was optimal to obtain the linear range of amplification.

Gene	Length of amplificate	Forward and reverse primer	Annealing temperature	Number of amplification cycles (*x* times)
PTP1B	701	5′-GAT GGA GAA GGA GTT CGA GGA G-3′ 5′-CCA TCA GTA AGA GGC AGG TGT C-3′	59.2	30
PPARγ	348	5′-GAG TCT GTG GGG ATA AAG CAT C-3′ 5′-CTC CAG GAC TCC TGC ACA T-3′	57.6	31
PEPCK	700	5′-AGC CTT TGG TCA ACA ACT GG-3′ 5′-CTA CGG CCA CCA AAG ATG AT-3′	54.3	27
F-1, 6-Dptase	447	5′-GTC AAC TGC TTC ATG CTG GA-3′ 5′-CCA CCA CCC TGT TGC TGT AG-3′	57.0	26
GAPDH	303	5′-ACG GGA AGC TCA CTG GCA TG-3′ 5′-CCA CCA CCC TGT TGC TGT AG-3′	cf. gene-specific temperatures	26

The PCR reactions for the amplification of fragments from the coding sequence of the genes examined were carried out in a reaction volume of 50 μl with a standard program for the single cycles. The standard program was as follows: initial denaturation: (95°C, 3 min) 1×; amplification cycles: (denaturation: 95°C, 45 s; annealing: primer-specific temperature, 40 s; extension: 72°C, 55 s) *x* times; final extension: (72°C, 5 min) 1×.

The further conditions in a typical 50-μl reaction were as follows:

10× PCR buffer (20 mM MgCL from MBI Fermentas)	5.00 μl
Taq Polymerase (Invitek)	0.04 U/μl
Mixed dNTPs (2 mM)	3.80 μl
Primer forward (10 pmol/μl)	2.50 μl
Primer reverse (10 pmol/μl)	2.50 μl
Nuclease free water	36.20 μl

The GAPDH fragment was amplified for 26 cycles.

Six microlitres of the amplification products was separated by electrophoresis in 1.5% agarose gels containing 0.1 μg ethidium bromide per millilitre. A molecular weight marker (Gene Ruler 100-bp DNA Ladder Plus #SM0321 from MBI Fermentas) was carried along in the gels. Additionally, the expression of the abovementioned genes was also examined in RNA samples obtained from three age- and sex-matched nondiabetic Black 6 mice. The gels were photographed under UV light with a gel imager (Gene Flash from Syngene), and optical density was evaluated using the software for the Syngene Imager. The expression of the genes examined was normalised to GAPDH expression.

2.3.4. Determination of the gluconeogenic marker enzymes F-1,6-Dptase, pyruvate carboxylase, phosphoenolpyruvate caboxykinase

The activity of the gluconeogenic marker enzymes F-1,6-Dptase, pyruvate carboxylase (PC) and PEPCK was measured photometrically by standard assays coupled to NAD/NADP–NADH/NADPH [45–47].

2.3.5. Measurement of parameters of lipid metabolism

2.3.5.1. Extraction of crude lipids from the liver. For the extraction of crude lipids, firstly, 1:10 (w/v) homogenates were prepared in 0.154 mol/L NaCl under a N_2 atmosphere using 0.25 g of liver per sample. To each sample, 2.5 ml of a hexane/isopropanol (3:2) mixture containing 0.005% butylated hydroxytoluene was then added. The samples were vortexed for 1 min and incubated at room temperature for 1 h after gassing with N_2. After centrifugation at 4500 U/min for 15 min, the upper lipid-containing phase was collected in a dried and tared sealable glass tube. The remaining pellet and the lower aqueous phase were then extracted for a further hour using 2 ml of the hexane/isopropanol (3:2) mixture. After centrifugation at 4500 U/min for 15 min, the upper phase was also collected into the first lipid extract. Then the solvent was evaporated in a N_2 atmosphere at 45°C, and, finally, the samples were dried in a vacuum dryer for 2 h.

Lipid concentration was determined gravimetrically, and the lipids were resolved in 1 ml hexane and frozen at −20°C until further analysis.

2.3.5.2. Determination of triglyceride concentration in plasma and liver. The concentration of triglycerides in plasma and in liver lipid extracts was determined with a test kit (Fluitest TG) from Biocon (Bangalore, India). The accuracy of the method was checked with Qualitrol.

2.3.5.3. Determination of cholesterol concentration in plasma and liver lipid extracts. Cholesterol concentration in plasma and in liver lipid extracts was measured with a test kit (Fluitest CHOL) from Biocon (Bangalore, India). The accuracy of the method was checked with Qualitrol.

2.3.5.4. Determination of phospholipid concentration in the liver. The concentration of phospholipids in liver lipid extracts was measured with a test kit from Boehringer (Mannheim, Germany) after digestion of the samples and liberation of the phospholipid phosphorus in a mixture of 70% perchloric acid and 30% H_2O_2.

2.3.6. Determination of the protein concentration in liver homogenates

The protein content in liver homogenates was determined using a standard protocol [48].

2.3.7. Statistical analysis

Statistical analysis of the experimental data was performed using the statistical package SPSS 12.0 for Windows. A one-way analysis of variance was performed after ascertaining the normality of distribution (Kolmogorov–Smirnov test or Shapiro–Wilk test) and the homogeneity of variance (Levene test) of the experimental data. If both conditions were fulfilled, differences between means were evaluated using the Tukey test. If homogeneity of variance could not be ensured, differences between means were examined using the Dunnett T3 test. Differences between means were assumed as significant at an error probability of less than 5% ($P<.05$). For some results, a trend is shown when error probability was less than 10% ($P<.1$).

3. Results

3.1. Whole-body insulin sensitivity

Fig. 2 shows the results of the whole-body IST. The blood glucose concentrations after the insulin challenge in the experimental groups are given as a percentage obtained in the initial status before putting the mice on special dietary conditions. Selenate treatment kept insulin sensitivity at a comparable level as in the initial status.

In selenium-deficient mice, initial blood glucose concentration (time: 0 min) before insulin injection was 1.5 to 2 times higher than in the initial status and in the selenate-treated mice. The lowering of blood glucose concentration in these groups takes place at significantly higher mean blood glucose concentrations. The higher insulin resistance in selenium-deficient and selenite-treated mice is indicated by a steep rise in the blood glucose response curve towards the initial values after 120 min.

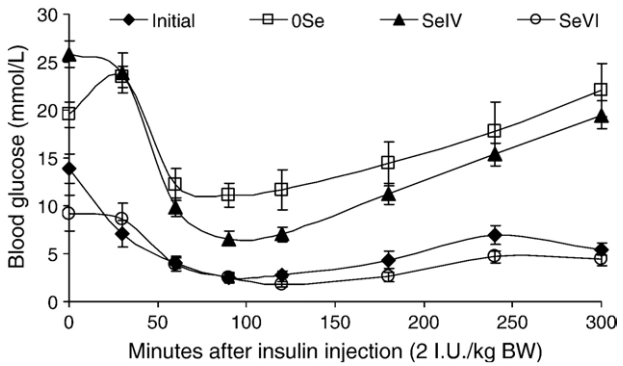


Fig. 2. Whole-body insulin sensitivity of *db/db* mice treated with selenate for 8 weeks in comparison to selenium-deficient and selenite-treated mice and to their initial status, obtained before putting the mice on defined dietary conditions. Each data point represents the mean±S.E.M. of seven animals per group.

Table 2
Parameters of the selenium- and glutathione-dependent cellular redox system: glutathione peroxidase 1 (mU/mg protein), thioredoxin reductase (mU/mg protein), glutathione reductase (mU/mg protein) and concentrations of total, oxidized and reduced glutathione in the liver of *db/db* mice treated with selenate for 8 weeks in comparison to selenium-deficient mice and selenite-treated mice (mean±S.D.)

Parameter of the selenium-and glutathione-dependent antioxidative system	0Se	SeIV	SeVI
Glutathione peroxidase 1	171 ± 25.9^{a}	703 ± 150^{c}	369 ± 43.1^{b}
Glutathione reductase	21.3 ± 2.27^{a}	17.2 ± 1.86^{a}	25.6 ± 4.54^{b}
Thioredoxin reductase	17.2 ± 9.77^{a}	53.1 ± 9.71^{b}	76.9 ± 24.9^{b}
Total glutathione	5.50 ± 0.49^{a}	6.69 ± 0.81^{b}	$6.24\pm0.97^{ab(<0.1)}$
Reduced glutathione	2.64 ± 0.32^{b}	2.13 ± 0.51^{a}	2.37 ± 0.51^{ab}
Oxidized glutathione	2.85 ± 0.27^{a}	4.56 ± 0.58^{b}	3.87 ± 0.49^{b}
% Reduced of total	48.03	31.67	37.71
% Oxidized of total	51.97	68.33	62.29

Significant differences ($P<.05$) within a row are indicated by different superscripts, "(<0.1)" shows a trend; $n=7$ animals per group considered for glutathione peroxidase and glutathione reductase; $n=6$ animals per group considered for glutathione- and thioredoxin reductase.

3.2. Selenium- and glutathione-dependent redox system

Eight weeks of supranutritional supplementation with selenite and selenate resulted in a significantly higher activity of liver GPx1 in selenite-treated and selenate-treated mice than in selenium-deficient mice. Thereby, it is noticeable that the selenate-treated mice had a significantly lower GPx1 activity than the mice with selenite application (Table 2).

The activity of both glutathione- and thioredoxin reductase was significantly increased by selenate treatment in comparison to the two other groups. With regard to the glutathione redox pair, the following measurements were made. Total glutathione concentration was slightly increased in the livers of both selenium-treated groups in comparison to the selenium-deficient group. This effect was significant between the selenite-treated group and the selenium-deficient group and in tendency between selenate-treated mice and selenium-deficient animals. The concentration of oxidized glutathione and the ratio of oxidized to total glutathione were increased by selenium treatment with both selenite and selenate; thus, the glutathione redox pair shifted to a more oxidized state. An attempt to mimic these characteristic changes of glutathione peroxidase and glutathione reductase activity in selenate-treated mice was made in vitro. As also found in the in vitro inhibition assay for PTPs (cf. Section 4.2), this in vitro assay provided evidence that in the intermediary metabolism of selenium compounds

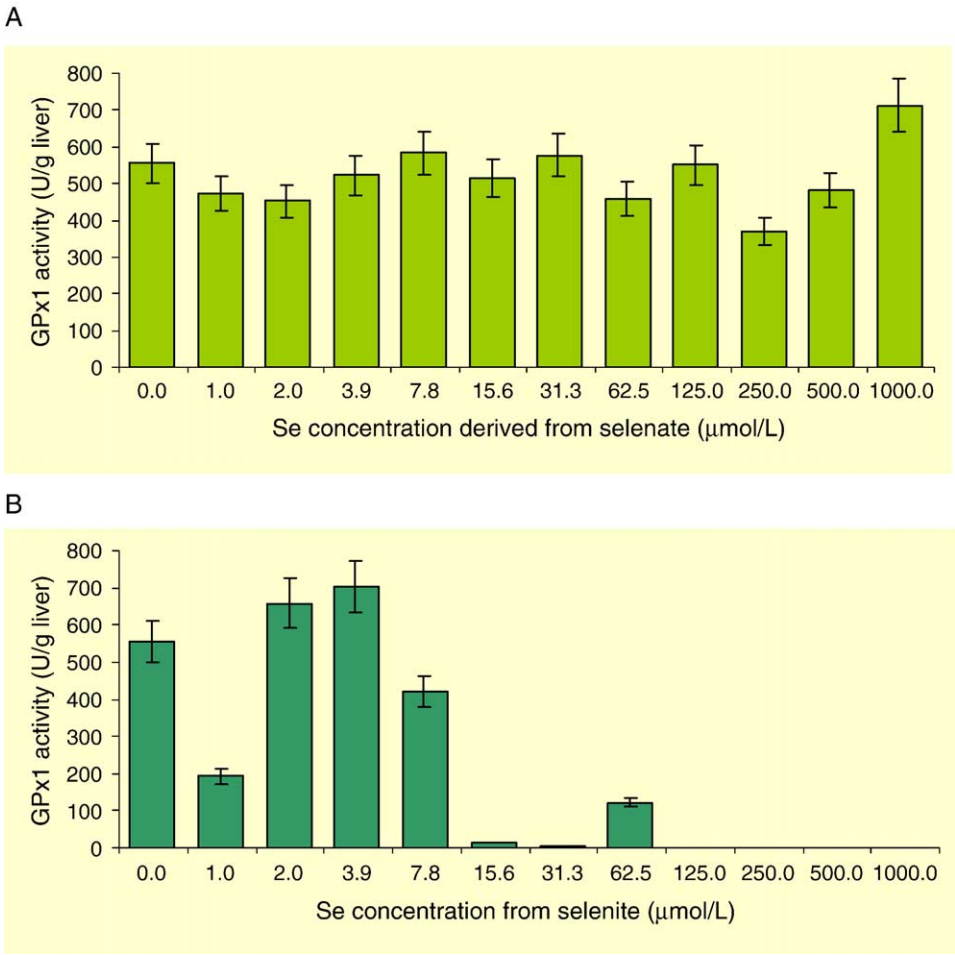


Fig. 3. Influence of different selenate (A) and selenite concentrations (B) on glutathione peroxidase activity in vitro. Each bar represents the mean±S.D. of three independent replications.

the selenite oxidation state +IV must be formed from selenate (oxidation state +VI) and is the actual biologically active metabolite.

The addition of increasing concentrations of selenate (0–1000 μmol/L) to the glutathione peroxidase assay (containing all components of the glutathione-dependent

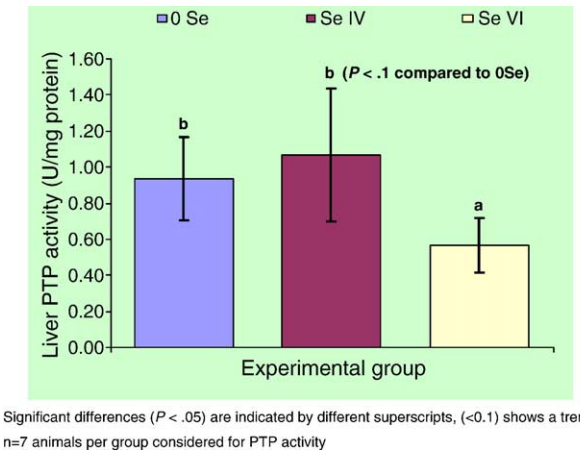


Fig. 4. Effect of 8 weeks of selenate treatment (SeVI) on the activity of PTPs (mean±S.D.) in the liver of *db/db* mice in comparison to selenium-deficient mice and selenite-treated mice.

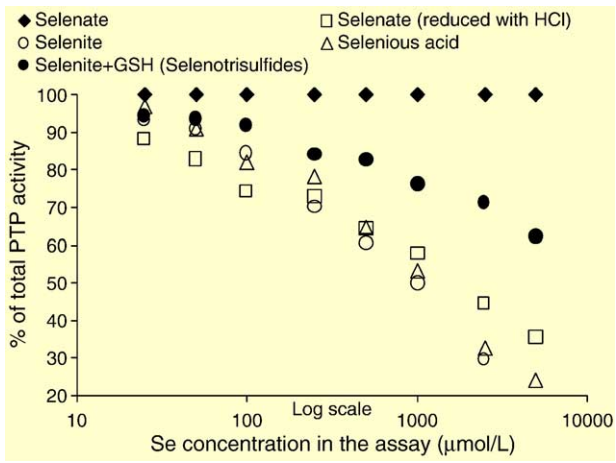


Fig. 5. In vitro test on the inhibition of PTPs by different selenium compounds and concentrations. Each data point represents the mean of three independent replications.

redox system) showed no overall influence on the activity of glutathione peroxidase activity (Fig. 3A). In contrast, the addition of selenite led to a reduction of glutathione peroxidase activity while the blank activity (glutathione reductase-dependent NADPH activity) increased. The addition of selenite concentrations above 62.5 μmol/L led to a total breakdown of the reaction (Fig. 3B).

3.3. Activity of PTPs as important antagonists of insulin signaling

Selenate treatment for 8 weeks inhibited the activity of PTPs as important antagonists of insulin signalling by about 50% as compared to selenium-deficient and selenite-treated animals (Fig. 4).

To ascertain the inhibitory effect of selenium compounds in different oxidation states on the activity of PTPs, an in vitro inhibition test was performed.

The addition of selenate (selenium oxidation state: +VI) to the reaction mixture reaching final selenium concentrations of 25, 50, 100, 250, 500, 1000, 2500 and 5000 μmol/L produced no inhibition of PTP activity. Adding selenium compounds of the oxidation state +IV, obtained either by nonenzymatic reduction of selenate with 37% HCl or by the addition of pure selenite or selenious acid to the reaction mixture, led to a strong inhibition of PTPs in a concentration-dependent manner (Fig. 5). A 50% inhibition of PTP activity was obtained with all the abovementioned selenium compounds of the oxidation state +IV when their final concentration in the assay mixture ranged between 500 and 1000 μmol/L. The inhibition of PTPs by tetravalent selenium compounds steadily increased up to the highest concentration examined in the test (5000 μmol/L Se), reaching a 65% inhibition by nonenzymatically reduced selenate and an 85% and 76% inhibition by the addition of pure selenite and selenious acid, respectively. Selenotrisulfides (oxidation state: −I), synthesized from selenite and reduced glutathione, effected a significantly lower inhibition of PTPs in comparison with the tetravalent selenium compounds. One hundred, 250, 500 and 1000 μmol/L of selenotrisulfides inhibited PTP activity only by 9%, 17%, 18% and 24%, respectively. Even with the addition of 5000 μmol/L selenotrisulfides to the reaction mixture the inhibition of PTPs remained below 50%.

Table 3
Activity of F-1,6-Dptase (U/mg protein), PC (U/mg protein) and PEPCK (U/mg protein) in the liver of *db/db* mice treated with selenate for 8 weeks in comparison to selenium-deficient and selenite-treated mice (mean±S.D.)

Gluconeogenic marker enzyme	0Se	SeIV	SeVI
Fructose-1,6-Dptase	0.640 ± 0.065^{b}	0.574 ± 0.014^{b}	0.237 ± 0.061^{a}
Pyruvate carboxylase	0.646 ± 0.059^{ab}	0.846 ± 0.220^{b}	0.595 ± 0.045^{a}
Phosphoenolpyruvate carboxykinase	0.306 ± 0.066^{b}	0.261 ± 0.069^{ab}	0.196 ± 0.051^{a}

Significant differences ($P<.05$) within a row are indicated by different superscripts; $n=6$ animals per group considered for the activities of F-1,6-Dptase, PC and PEPCK.

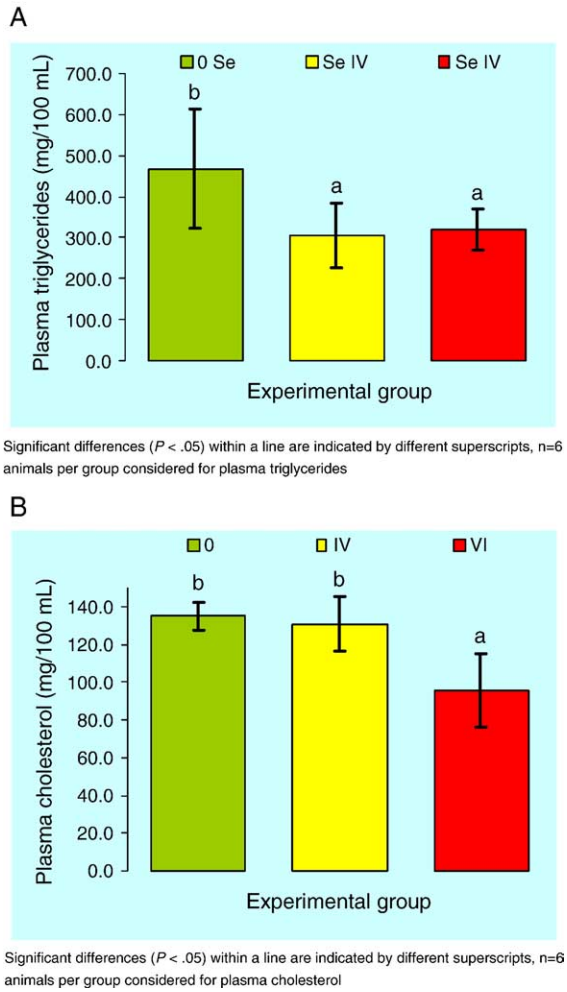


Fig. 6. Triglyceride concentration (mg/100 ml) (A) and cholesterol concentration (mg/100 ml) (B) in plasma of *db/db* mice treated with selenate for 8 weeks in comparison to selenium-deficient and selenite-treated mice (mean±S.D.).

The results of the PTP in vitro inhibition test provided further evidence that selenium compounds of the selenite oxidation state +IV must be formed intermediarily from selenate and actually mediate the biological properties of selenate in vivo.

Selenate administration to *db/db* mice led to a reduction of the activity of the gluconeogenic enzymes F-1,6-Dptase, PC and PEPCK in comparison to selenium-deficient mice and selenite-treated mice (Table 3).

3.4. Parameters of lipid metabolism

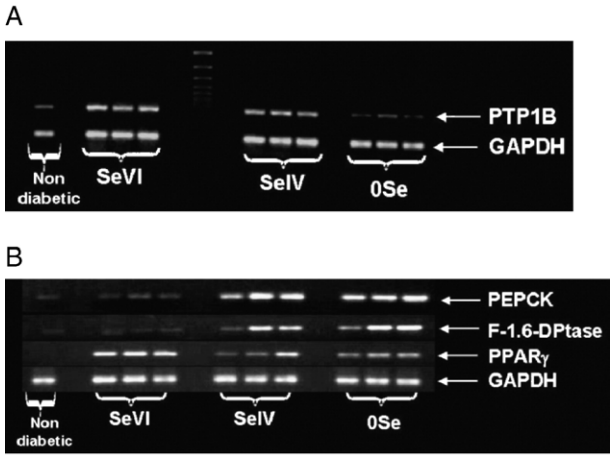
In comparison to selenium deficiency, plasma triglyceride concentration was significantly lowered by administration of both selenium compounds to *db/db* mice (Fig. 6A). Selenate treatment additionally lowered plasma cholesterol concentration in comparison to the two other experimental groups (Fig. 6B).

Treatment of *db/db* mice with selenite increased total liver lipid content per gram fresh matter in tendency, whereas treatment with selenate led to a significant increase in total liver lipids as compared to feeding with selenium-deficient diet (Table 4). No changes could be found for the content of phospholipids. A significantly higher cholesterol concentration was evident in selenite-treated mice than in selenium-deficient and selenate-treated mice.

When the lipid parameters were referred to 1 g of total lipids, all parameters measured were significantly lower in selenate-treated mice than in selenium-deficient and selenite-treated animals, whereas the remnant to 1 g of total lipids was significantly increased by selenate treatment, presumably indicating an increased concentration of free fatty acids.

3.5. Expression of genes related to insulin resistance, glucose metabolism and fatty acid metabolism

With regard to expression of genes related to insulin resistance, glucose metabolism and fatty acid metabolism, some marked changes could be measured. Selenium supplementation with selenite or selenate increased the expression of PTP1B, an important tyrosine phosphatase discussed in the context of insulin resistance, by about 2- or 2.5-fold in comparison to selenium deficiency (Fig. 7A). Selenate administration to the *db/db* mice led to a marked down-regulation of the gluconeogenic marker enzymes F-1.6-Dptase and PEPCK in comparison to selenium-deficient and selenite-treated mice. The expression reached a level almost as low as in nondiabetic Black 6 mice. The expression of the PPARγ as an efficient target in the treatment of obesity and insulin resistance, which is mainly expressed in adipose tissue, but also in the liver of obese rodents, was about 2.5-fold increased in the liver of selenate-treated mice in comparison to their selenium-deficient and selenite-treated companions. Under the conditions examined (up to 31 amplification cycles), no expression of PPARγ could be detected in nondiabetic Black 6 mice (Fig. 7B).



n=6 (3 pools of 2 animals per group) considered for the expression experiments

Fig. 7. Expression of PTP1B (A), PEPCK, F-1.6-Dptase and PPARγ (B) in the liver of *db/db* mice treated with selenate for 8 weeks in comparison to mice on selenium-deficient diet, mice treated with selenite and nondiabetic Black 6 control mice relative to their respective GAPDH expression.

Table 4
Parameters of lipid metabolism: total lipids, triglycerides, phospholipids and cholesterol in the liver of *db/db* mice treated with selenate for 8 weeks in comparison to selenium-deficient mice and selenite-treated mice based on 1 g of liver fresh matter and 1 g of total lipids, respectively (mean±S.D.)

Parameter of liver fatty acid metabolism	0 Se	Se IV	Se VI
Parameters referring to 1 g of liver fresh matter			
Total lipids (mg/g fresh matter)	$79.6\pm7.14^{a(<.1)}$	99.4 ± 16.4^{b}	145 ± 26.9^{c}
Triglycerides (mg/g fresh matter)	36.3 ± 22.1^{a}	$62.7\pm10.6^{b(<.1)}$	$71.0\pm24.0^{b(<.1)}$
Phospholipids (mg/g fresh matter)	18.7 ± 1.19^{a}	18.6 ± 2.16^{a}	19.0 ± 1.32^{a}
Cholesterol (mg/g fresh matter)	3.30 ± 0.64^{a}	4.87 ± 1.00^{b}	3.35 ± 1.13^{a}
Parameters referring to 1 g of liver lipids			
Triglycerides (mg/g lipids)	633 ± 80.9^{a}	639 ± 97.4^{a}	$491\pm150^{b(<.1)}$
Phospholipids (mg/g lipids)	236 ± 20.8^{c}	190 ± 31.5^{b}	138 ± 35.7^{a}
Cholesterol (mg/g lipids)	43.6 ± 7.25^{b}	50.3 ± 11.91^{b}	24.1 ± 10.24^{a}
Rest to 1 g lipids (mg)	87.4	120.7	346.9

Significant differences ($P<.05$) within a row are indicated by different superscripts; "(<0.1)" shows a trend; $n=6$ animals per group considered for liver lipid parameters.

4. Discussion

In the present study, treatment of the *db/db* mice with supranutritional selenate doses effected an improvement of whole-body insulin sensitivity in comparison to selenium-deficient and selenite-treated mice by maintaining insulin sensitivity on a comparably low level as at the beginning of the trial.

4.1. Selenium- and glutathione-dependent redox system

Final GPx1 activity in the liver clearly indicated an efficient selenium depletion in group 0Se. GPx1 activity in the selenium-supplemented groups SeIV and SeVI, on the one hand, reflected a high selenium status in these organs, but, on the other hand, the distinctly lower GPx1 activity in the liver of selenate-treated mice demonstrates that fundamental differences exist in the absorption and the intermediary metabolism of selenite and selenate. The results of the in vitro assay on the influence of selenite and selenate on GPx1 activity strongly suggest that the selenite oxidation state +IV is intermediarily formed from selenate and acts as an inhibitor of GPx1.

Our results with regard to a distinctly different metabolism for selenite and selenate are confirmed by

prior investigations on selenium absorption and by studies characterising intermediary selenium metabolites [9,10,13,14].

From the higher activity of both glutathione reductase and thioredoxin reductase, in particular with selenate treatment, it can be concluded that these reductases are of significance in the reduction of selenate, since selenate must be reduced from the oxidation state +VI to the oxidation state −II, while selenite metabolites (selenotrisulfides) depend only on one reduction step from −I to −II (cf. Fig. 1).

In the present study, the administration of both selenite and selenate further effected a moderate increase in total glutathione concentration and a significant change in the ratio of oxidized to reduced glutathione. Similar findings were reported for a feeding trial with rats and ducklings and could be caused by the enhanced need for reduced glutathione for selenium reduction to the oxidation state −II and a limited capacity of glutathione reductase when supranutritive selenium concentrations are administered to animals [49,50]. Moreover, an increase in oxidized glutathione was reported for rats fed fish oil-enriched diets supplemented with selenium in accordance with dietary recommendations and for rats fed selenium from different compounds in various concentrations [51]. The effects of changes in cellular redox status on insulin resistance have been controversially discussed. On the one hand, a decrease in tissue GSH concentrations by treating rats with the γ-glutamyl cysteinyl synthetase inhibitor buthionine sulfoximine led to a significant impairment of insulin sensitivity of cultured adipocytes from these animals [52]. On the other hand, results from tissue culture studies with α-lipoic acid showed that the short-term enhancement of intracellular oxidant levels led to an enhanced glucose uptake [53]. Further, the effectiveness of pentavalent vanadium, acting as a PTP1B inhibitor on insulin sensitivity, was enhanced in the presence of higher oxidant levels in cells, because under these conditions the reduction of the more effective pentavalent vanadium to less efficient tetravalent vanadium was delayed [54].

4.2. Activity and expression of PTPs as important antagonists of insulin signalling and particular changes in glucose metabolism

A significant decrease of PTP activity in the liver was obtained by oral selenate administration alone. Supranutritional doses of both selenite and selenate increased the expression of PTP1B.

Within the PTPs, a 230-amino acid domain, which includes the active centre of the enzymes, is a highly conserved region in the protein structure. A cysteine residue in this region is involved in the hydrolysis of protein phosphotyrosine residues by the formation of a cysteinyl-phosphate intermediate [55]. In recent years, PTP1B, which is involved in the negative regulation of insulin signalling, has been of particular interest. At present, the expression and activity of PTP1B in rodents and other mammalian species are controversially discussed. However, there is no doubt that diabetic symptoms can be efficiently reduced by treatment with PTP1B enzyme inhibitors or antisense oligonucleotides, which reduce the mRNA expression and the protein synthesis of the enzyme [56–59]. Explanations of the mechanism for reversible and irreversible PTP1B inhibition by glutathionylation in the presence of high concentrations of oxidized glutathione or formation of sulphenic, sulphinic and sulphonic acid derivatives in the presence of hydrogen peroxide have been given involving the blocking as well as the stepwise oxidation of the active site cysteine SH group. Even in vivo, an insulin-dependent release of hydrogen peroxide in tissues leads to an oxidation of PTP1B and an increase in insulin signalling [60–63]. Furthermore, the results of a recent study in mice with GPx1 overexpression support the hypothesis of a differentiated regulation of PTPs by pro- and antioxidative metabolites. In this study, mice with GPx1 overexpression showed a diminished phosphorylation of the β subunit of the insulin receptor [64]. This effect can be explained by a reduced oxidation (inactivation) of the active site of PTPs by reduced hydrogen peroxide levels; thus, the enzymes possess a higher activity towards the phosphorylated β subunit of the insulin receptor, and, therefore, insulin resistance increases. An increased concentration of oxidized glutathione as a result of the increased GPx1 activity, however, may produce higher amounts of glutathionylated PTPs (inactive), leading to an up-regulation of their mRNA and activity, and, finally, effecting an enhanced dephosphorylation of the β subunit of the insulin receptor [64]. From the results of this study and from further unpublished results, we conclude that glutathionylation of PTPs due to a high GPx1 activity and a shift of the glutathione redox pair to a more oxidized state is the driving force for the increased expression of PTP1B.

The in vitro inhibition assay was performed to investigate whether the observed PTP reduction by selenate in vivo is based on the inhibition of these enzymes. Initially, surprisingly and contrary to the in vivo results, the incubation of *db/db* mouse liver cytosol with increasing selenate concentrations (Se oxidation state +VI) effected no inhibition of PTPs, whereas the incubation with SeIV compounds obtained either by nonenzymatic reduction of selenate or by the addition of pure SeIV derivatives (selenite and selenious acid) led to a concentration-dependent inhibition of PTP activity (Fig. 5). As for glutathione peroxidase activity (cf. Section 4.1), a possible explanation for this observation that free SeIV compounds act as the actual inhibitors of PTPs could be derived from the differences in the metabolism of selenate and selenite in vivo (Fig. 1).

In mammals, selenate is absorbed unmodified by a sodium-dependent cotransport system, which is also involved in sulphate absorption [10]. Furthermore, there is evidence that selenate is distributed unmodified to peripheral tissues, where it is stepwise reduced to the oxidation state −II (Fig. 1). The exact effect of selenate

metabolism in mammalian tissues is not clear yet [14]. Either selenate is metabolised in a similar manner to sulfate or it undergoes reduction during which the oxidation state +IV is formed as an intermediate. Due to the reduction of PTP activity observed in selenate-treated mice, the latter mentioned pathway seems to play an important role.

In contrast to selenate, most of the selenite does not reach the peripheral tissues in the oxidation state +IV since selenite forms selenotrisulfides (oxidation state: −I) during its intestinal absorption. These selenotrisulfides are distributed to organs on the periphery and undergo reduction to the oxidation state −II, from which they can be utilized for the synthesis of functional selenoproteins [10,13]. This hypothesis is supported by the results from the in vitro inhibition test. When selenite and GSH were converted to selenotrisulfides prior to their use in the in vitro inhibition test, the inhibition of PTPs decreased significantly. The remaining inhibition may have derived from a not fully completed synthesis of selenotrisulfides in the model investigated. This must be examined in future investigations with purified selenotrisulfides and pure preparations of PTP1B. The precise inhibition mechanism of SeIV compounds on PTPs in general and on PTP1B in particular seems to be an interesting subject for future investigations using mass spectrometry.

In conclusion, our results on PTP activity and expression can be interpreted as shown in Fig. 8.

High supranutritional selenium doses effect a shift in the glutathione–redox system to a more oxidized state. An enhanced glutathionylation of PTPs is presumably the stimulus for an increase in gene expression.

In the case of very high selenate doses, the inhibitory effect of intermediary selenate metabolites compensates for the increased expression, which, in turn, leads to a correction of insulin signalling and particular changes in the intermediary glucose metabolism. In particular, a reduction of the activity and the expression of gluconeogenic marker enzymes was obtained (Fig. 8). In prior studies with type I diabetic rats, the regulatory effect of selenate on glycolysis and gluconeogenesis was attributed to an increase in the phosphorylation of single components of the insulin signalling pathway. From our present results, we now conclude that the inhibition of PTPs is the cause of the modification of insulin signalling.

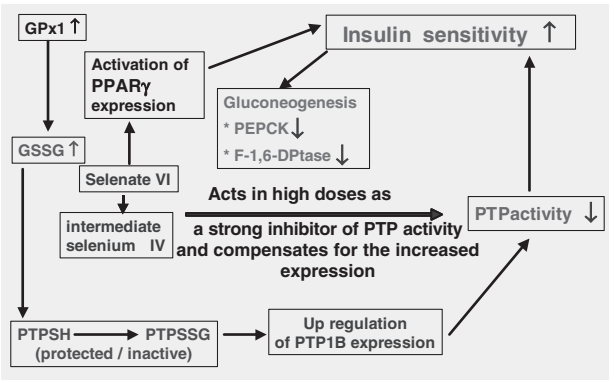


Fig. 8. Possible links between the selenium-dependent redox system, the regulation of PTPs and the resulting influence on glucose metabolism.

Future investigations into the precise regulation of PTP1B mRNA expression and PTP1B protein expression should focus on the role of cellular redox status during these processes.

4.3. Influence on fatty acid metabolism

With regard to fatty acid metabolism, supranutritional selenate led to a significant decrease in plasma cholesterol and triglycerides. Concomitantly, a significant increase in total liver lipid concentration, liver triglyceride concentration and expression of PPARγ was measured.

The main functions of PPARγ consist of adipocyte differentiation and the redistribution of adipose tissue. Furthermore, PPARγ seems to be involved in the distribution of body fat stores. These hypotheses were confirmed by a study with transgenic mice with an ablation of liver PPARγ but with adipose tissues. However, these animals developed fat intolerance, increased adiposity, hyperlipidemia and insulin resistance. It could be concluded that liver PPARγ regulates triglyceride homeostasis, contributing to hepatic steatosis, but protecting other tissues from triglyceride accumulation and insulin resistance [32,33]. Results of other trials in which *db/db* mice were long-term treated with thiazolidinediones showed that these insulin-sensitising pharmaceuticals induced liver PPARγ and its target genes (adipocyte FABP) [34], finally resulting in hepatic steatosis [35,36].

Our study shows that a similar mechanism seems to be activated by selenate. Selenate-treated *db/db* mice gained far less body weight than their selenium-deficient and selenite-treated companions [29]; they showed reduced plasma lipids and a distinct increase in liver lipids. The lipid fractions per gram of total lipids indicate that in selenate-treated *db/db* mice the “rest” to 1 g of total lipids is significantly higher than in the two other groups and therefore demonstrate a higher amount of nonesterified fatty acids. These nonesterified fatty acids in turn can act as natural ligands of PPARγ and therefore contribute to an increase in whole-body insulin sensitivity (Fig. 8).

5. Conclusion

The results of our study with type II diabetic *db/db* mice give some new insight into the mechanisms by which the administration of supranutritional selenate can influence diabetes and insulin resistance. One mechanism of interest is the inhibition of PTPs by intermediary selenate metabolites. This aspect of an antidiabetic action is closely linked to selenium metabolism, since selenium metabolites in the oxidation state +IV are the actual inhibitors of PTPs and they can be generated only from the stepwise

reduction of selenate. This is likewise the reason why selenite fails to develop strong antidiabetic properties, because this selenium compound enters peripheral organs in the selenotrisulfide oxidation state −I. Furthermore, we could demonstrate that the system of PPARs is also initiated by supranutritional selenate. The increased expression of liver PPARγ presumably led to a redistribution of whole-body lipid stores resulting in an increase in liver lipids. In turn, the concentration of lipids in the liver can provide natural ligands of PPARγ and therefore contribute to the increase in insulin sensitivity.

Acknowledgment

We thank Dipl. Biol. Sandra Schneider and Prof. Dr. R. Schmidt from the Biotechnical Centre of Giessen University for their advice in the RT-PCR experiments. Further thanks are addressed to our Bachelor and Masteral students Linda Minke and Jenny Schaefer for their help with analyses within the scope of their Bachelor and Masteral theses.

For financial support, we thank the H.W. Schaumann Foundation for Agricultural Sciences (Hamburg, Germany).

References

[1] NRC (National Research Council). Nutrient requirements of laboratory animals. 4th revised ed. Washington (DC): National Academy Press; 1995.
[2] NRC (National Research Council). Nutrient requirements of poultry. 9th revised ed. Washington (DC): National Academy Press; 1994.
[3] NRC (National Research Council). Nutrient requirements of sheep. 6th revised ed. Washington (DC): National Academy Press; 1995.
[4] NRC (National Research Council). Nutrient requirements of swine. 10th revised ed. Washington (DC): National Academy Press; 1998.
[5] NIH (National Institute of Health) (Updated: 08/01/2004): http://ods.od.nih.gov/factsheets/selenium.asp.
[6] Brigelius-Flohé R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 2000;27:951–65.
[7] Köhrle J. Thyroid hormone deiodinases — a selenoenzyme family acting as gate keepers to thyroid hormone action. Acta Med Austriaca 1996;23:17–30.
[8] May JM, Mendiratta S, Hill KE, Burk RF. Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. J Biol Chem 1997;272:22607–10.
[9] Wolffram S, Berger B, Grenacher B, Scharrer E. Transport of selenoamino acids and their sulfur analogues across the intestinal brush border membrane of pigs. J Nutr 1989;119:706–12.
[10] Wolffram S, Arduser F, Scharrer E. In vivo intestinal absorption of selenate and selenite by rats. J Nutr 1985;115:454–9.
[11] Beilstein MA, Whanger PD. Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine. J Nutr 1986; 116:1711–9.
[12] Beilstein MA, Whanger PD. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. J Nutr 1986;116:1701–10.
[13] Suzuki KT, Shiobara Y, Itoh M, Omichi M. Selective uptake of selenite by red blood cells. Analyst 1998;123:63–7.
[14] Shiobara Y, Ogra Y, Suzuki KT. Speciation of metabolites of selenate in rats by HPLC-ICP-MS. Analyst 1999;124:1237–41.
[15] Sunde RA, Evenson JK. Serine incorporation into the selenocysteine moiety of glutathione peroxidase. J Biol Chem 1997;262:933–7.
[16] Böck A, Forchhammer K, Heider J, Leinfelder W, Sawers G, Veprek B, et al. Selenocysteine: the 21st amino acid. Mol Microbiol 1991; 5:515–20.
[17] Park SI, Park JM, Chittum HS, Yang ES, Carlson BA, Lee BJ, et al. Selenocysteine tRNAs as central components of selenoprotein biosynthesis in eukaryotes. Biomed Environ Sci 1997;10:116–24.
[18] Ezaki O. The insulin-like effect of selenate in rat adipocytes. J Biol Chem 1990;262:6658–62.
[19] Furnsinn C, Englisch R, Ebner K, Nowotny P, Vogle C, Waldhausl W. Insulin-like vs. non-insulin stimulation of glucose metabolism by vanadium tungsten and selenium compounds in rat muscle. Life Sci 1996;59:1989–2000.
[20] McNeill JH, Delgatty HL, Battell ML. Insulinlike effects of sodium selenate in streptozotocin-induced diabetic rats. Diabetes 1991;40: 1675–8.
[21] Battell ML, Delgatty HL, McNeill JH. Sodium selenate corrects glucose tolerance and heart function in STZ diabetic rats. Mol Cell Biochem 1998;179:27–34.
[22] Berg EA, Wu JY, Campbell L, Kagey M, Stapleton SR. Insulin-like effects of vanadate and selenate on the expression of glucose-6-phosphate dehydrogenase and fatty acid synthase in diabetic rats. Biochimie 1995;77:919–24.
[23] Stapleton SR, Jivraj S, Wagle A. Insulin and the insulin-mimetic selenium mediate the regulation of glucose-6-phosphate-dehydrogenase gene expression via different signal proteins. FASEB J 1998; 12:1404.
[24] Becker DJ, Reul B, Ozcelikay AT, Buchet JP, Henquin JC, Brichard SM. Oral selenate improves glucose homeostasis and partly reverses abnormal expression of liver glycolytic and gluconeogenic enzymes in diabetic rats. Diabetologia 1996;39:3–11.
[25] Stapleton SR, Garlock GL, Foellmi-Adams L, Kletzien RE. Selenium potent stimulator of tyrosyl phosphorylation and activator of MAP kinase. Biochim Biophys Acta 1997;1355:259–69.
[26] Hei YJ, Farahbakshian S, Chen X, Battel ML, McNeill JH. Stimulation of MAP kinase and S6 kinase by vanadium and selenium in rat adipocytes. Mol Cell Biochem 1998;178:367–75.
[27] Sheng XQ, Huang KX, Xu HB. New experimental observation on the relationship of selenium and diabetes. Biol Trace Elem Res 2004; 99:241–53.
[28] Mueller AS, Pallauf J, Rafael J. The chemical form of selenium affects insulinomimetic properties of the trace element: investigations in type II diabetic *dbdb* mice. J Nutr Biochem 2003;14:637–47.
[29] Mueller AS, Most E, Pallauf J. Effects of a supranutritional dose of selenate compared to selenite on insulin sensitivity in type II diabetic *dbdb* mice. J Anim Physiol Anim Nutr 2005;89:94–104.
[30] Ferre P. The biology of peroxisome proliferator-activated receptors. Relationship with lipid metabolism and insulin sensitivity. Diabetes 2004;53:S43–S50.
[31] Vidal-Puig A, Jimenez-Linan M, Lowell BB, Hamann A, Hu E, Spiegelman B, et al. Regulation of PPARγ gene expression by nutrition and obesity in rodents. J Clin Invest 1996;97:2553–61.
[32] Schadlinger SE, Bucher NE, Schreiber BM, Farmer SR. PPARγ2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes. Am J Physiol Endocrinol Metab 2005;288:E1195–205.
[33] Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, et al. Liver peroxisome proliferator-activated receptor γ contributes to hepatic steatosis, triglyceride clearance, and the regulation of body fat mass. J Biol Chem 2003;278:34268–76.
[34] Memon RA, Tecott LH, Nonogaki K, Beigneux A, Moser AH, et al. Upregulation of peroxisome proliferator activated receptors (PPAR-alpha) and PPAR-gamma messenger ribonucleic acid expression in the liver in murine obesity: troglitazone induces expression of PPAR-gamma-responsive adipose tissue specific genes in the liver of obese diabetic mice. Endocrinology 2000;141:4021–31.
[35] Burant CF, Sreenan S, Hirano K-I, Tai TAC, Lohmiller J, et al. Troglitazone action is independent of adipose tissue. J Clin Invest 1997;100:2900–8.

[36] Chao L, Marcus-Samuels B, Mason MM, Moitra J, Vinson C, et al. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. J Clin Invest 2000;106: 1221–8.
[37] Tappel ME, Chaudiere J, Tappel AL. Glutathione peroxidase activities of animal tissues. Comp Biochem Physiol 1982;73 [B]:945–9.
[38] Cohen MB, Duvel DL. Characterization of the inhibition of glutathione reductase and the recovery of enzyme activity in exponentially growing murine leukemia (L1210) cells treated with 1,3-bis(2-chloroethyl)-1-nitrosourea. Biochem Pharmacol 1988;37: 3317–20.
[39] Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 1982; 106:207–12.
[40] Gromer S, Arscott LD, Williams CH, Schirmer RH, Becker K. Human placenta thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics, and inhibition by therapeutic gold compounds. J Biol Chem 1998;273:20096–101.
[41] Zhu L, Goldstein B. Use of an anaerobic chamber environment for the assay of endogenous cellular protein tyrosine phosphatase activities. Biol Proced Online 2002;4:1–9.
[42] Montalibet J, Skorey KI, Kennedy BP. Protein tyrosine phosphatase: enzymatic assays. Methods 2005;35:2–8.
[43] Ganther HE. Selenotrisulfides. Formation by the reaction of thiols with selenious acid. Biochemistry 1968;7:2898–905.
[44] Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidiniumthiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.
[45] Pontremoli P, Melloni E. Fructose-1.6-diphosphatase from rabbit liver. Methods Enzymol 1975;42:354–9.
[46] Warren GB, Tipton KF. Pig liver pyruvate carboxylase. Purification, properties and cation specificity. Biochem J 1976;139:297–310.
[47] Wimonwatwatee T, Heydari AR, Wu WT, Richardson A. Effect of age on the expression of phosphoenolpyruvate carboxykinase in rat liver. Am J Physiol Gastrointest Liver Physiol 1994;267: G201–4.
[48] Bradford MM. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[49] Thompson HJ, Ip C. Temporal changes in tissue glutathione in response to chemical form, dose, and duration of selenium treatment. Relevance to cancer chemoprevention by selenium. Biol Trace Elem Res 1991;30:163–73.
[50] Hoffmann DJ, Heinz GH, LeCaptain LJ, Eisemann JD, Pendleton GW. Toxicity and oxidative stress of different forms of organic selenium and dietary protein in mallard ducklings. Arch Environ Contam Toxicol 1996;31:120–7.
[51] Schäfer K, Kyriakopoulos A, Gessner H, Grune T. Effects of selenium deficiency on fatty acid metabolism in rats fed fish oil-enriched diets. J Trace Elem Med Biol 2004;18:89–97.
[52] Khamaisi M, Kavel O, Rosenstock M, Porat M, Yuli M, Kaiser N, et al. Effect of inhibition of glutathione synthesis on insulin action: in vivo and in vitro studies using buthionine sulfoximine. Biochem J 2000;349(Pt 2):579–86.
[53] Cho KJ, Moini H, Shon HK, Chung AS, Packer L. Alpha-lipoic acid decreases thiol reactivity of the insulin receptor and protein tyrosine phosphatase 1B in 3T3-L1 adipocytes. Biochem Pharmacol 2003;66: 849–58.
[54] Lu B, Ennis D, Lai R, Bogdanovic E, Nikolov R, Salamon L, et al. Enhanced sensitivity of insulin-resistant adipocytes to vanadate is associated with oxidative stress and decreased reduction of vanadate (+5) to vanadyl (+4). J Biol Chem 2001;276(38):35589–98.
[55] Denu JM, Dixon JE. Protein tyrosine phosphatases: mechanisms of catalysis and regulation. Curr Opin Chem Biol 1998;2:633–41.
[56] Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, et al. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci U S A 2002;99:11357–62.
[57] Gum RJ, Gaede LL, Koterski SL, Heindel M, Clampit JE, Zinker BA, et al. Reduction of protein tyrosine phosphatase 1B increases insulin-dependent signaling in *ob/ob* mice. Diabetes 2003;52:21–8.
[58] Ramachandran C, Kennedy BP. Protein tyrosine phosphatase 1B: a novel target for type 2 diabetes and obesity. Curr Top Med Chem 2003;3:749–57.
[59] Harley EA, Levens N. Protein tyrosine phosphatase 1B inhibitors for the treatment of type 2 diabetes and obesity: recent advances. Curr Opin Investig Drugs 2003;4:1179–89.
[60] Salmeen A, Andersen JN, Myers MP, Meng TZ, Hinks JA, Tonks NK, et al. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. Nature 2003;423:769–73.
[61] Van Montfort RL, Congreve M, Tisi D, Carr R, Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. Nature 2003;423:773–7.
[62] Barrett WC, DeGnore JP, Koenig S, Fales HM, Keng YF, Zhang ZY, et al. Regulation of PTP1B via glutathionylation of the active site cysteine 215. Biochemistry 1999;28:6699–705.
[63] Mahadev K, Zilbering A, Zhu L, Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1B in vivo and enhances the early insulin action cascade. J Biol Chem 2001;276:21938–42.
[64] McClung JP, Ronecker CA, Weipeng M, Lisk DJ, Langlais P, Liu F, et al. Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. Proc Natl Acad Sci U S A 2004;101:8852–7.

Selenium, an Ambivalent Factor in Diabetes? Established Facts, Recent Findings and Perspectives

Andreas S. Mueller, Astrid Bosse and Josef Pallauf*

Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, Heinrich Buff Ring 26 - 32, D-35392 Giessen, Germany

Abstract: It is widely known that selenium develops its biological activity *via* an active selenocysteine residue in the catalytically active centre of functional selenoproteins. By its function in glutathione peroxidases and thioredoxin reductases selenium contributes to a remarkable extent to the maintenance of the cellular antioxidative balance when taken up at the recommended dietary level (animals: 0.1 - 0.3 mg/kg diet, humans: 50 - 150 µg Se daily). In recent years an interesting physiological aspect has been found for selenate (selenium oxidation state +VI). High doses of selenate displayed antidiabetic properties when applied to diabetic animals or added to the media of tissue cultures. Thus selenate treatment could be shown to normalise hyperglycaemia as well as changed activities of glycolytic and gluconeogenic marker enzymes. Mechanistically an increased phosphorylation of single proteins of the insulin signalling cascade could be attributed to the insulinomimetic action of selenate.

The examination of the antidiabetic features of selenate in type II diabetic animals revealed that the increase in phosphorylation is presumably based on the inhibition of protein tyrosine phosphatases, which act as negative regulators of insulin signalling.

In contrast to the antidiabetic features of high selenate doses, selenite administration to diabetic animals showed no effect on diabetes. In a recent study it could even be demonstrated that the overexpression of glutathione peroxidase 1 (the best characterized selenoprotein) in healthy mice led to an increase in insulin resistance and obesity.

These results could partially be confirmed by the data of our most recent investigation in which a high expression and activity of glutathione peroxidase, obtained by feeding selenium at the nutritionally recommended level and at a moderately supranutritive level corresponded to an up-regulated expression of proteins whose expression is increased in insulin resistant type 2 diabetes.

From studies on the role of selenium in diabetes carried out so far it can be concluded that selenium plays an ambivalent role with regard to diabetes depending on the compound and on the applied concentration. Thus only high doses of selenate evolve antidiabetic properties. Investigations into an even negative influence of moderate supranutritive doses of selenium on diabetes and the molecular events linked to this are necessary.

The review summarizes the information currently available on the ambivalent role of selenium in diabetes which seems to depend on the chemical form and the applied concentration. Established facts, recent findings of our own studies using microarray analysis and RT-PCR and perspectives of the role of selenium in diabetes are presented and discussed against the background of selenium metabolism.

Keywords: Selenium, selenate, selenite, antidiabetic effects and insulin resistance.

1. SELENIUM AND SELENIUM COMPOUNDS IN MAMMALS

Selenium belongs to the chalcogens and many of its chemical properties (outer valence shell electronic configuration, atomic size, bond energy, ionisation potential, electronegativity) are similar to those of sulphur. As in the case of sulphur selenium occurs in the oxidation states –II (selenide), 0 (elemental selenium), +IV (selenite), +VI (selenate). In its elemental oxidation state (0) selenium forms red crystals with a hexagonal ring structure [1]. Besides these similarities there exist nevertheless some important differences between the two elements with regard to the chemistry of their oxyanions and in the acid strengths of their hydrides. Since Se compounds tend to be metabolised to more reduced states and sulphur compounds tend to be metabolised to more oxidized states, the following reaction between quadrivalent selenium and quadrivalent sulphur is the basis for many reactions in biological systems:

$H_2SeO_3 + H_2SO_3 \rightarrow Se + 2\ H_2SO_4 + H_2O$ [2, 3].

When taken up at the recommended level (animals: 0.15 – 0.30 mg Se/kg dietary dry matter, humans: 50 – 150 µg Se daily) [4, 5, 6, 7, 8, 9] selenium performs its physiological functions in the body of animals and humans as an integral

*Address correspondence to this author at the Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, Heinrich Buff Ring 26 - 32, D-35392 Giessen, Germany; E-mail: Josef.Pallauf@ernaehrung.uni-giessen.de

1573-4013/06 $50.00+.00

part of the redox-active centre of functional selenoproteins. The detoxification of peroxides, the involvement in the regulation of thyroid hormone metabolism and the participation in the reduction of disulfides and ascorbate are the most important functions fulfilled by at least four different glutathione peroxidases, three iodothyronine deiodinases and two thioredoxin reductases [10, 11, 12]. Within the glutathione peroxidases GPx1 is the glutathione peroxidase which normally exhibits the highest activity and is expressed in all mammalian tissues. Presumably GPx1 makes a large contribution to the maintenance of cellular antioxidant balance by the detoxification of a broad variety of peroxides and hydroperoxides as shown in Fig. (**1**) [13].

GPx2, the gastrointestinal form, has been assumed to protect the organism against food borne hydroperoxides though without convincing evidence. Its peculiar cellular and subcellular distribution indicate more specific functions such as regulating proliferation and apoptosis of the gastrointestinal epithelium [14]. GPx3 detoxifies peroxides using glutaredoxin and thioredoxin caused by a lack of reduced glutathione in blood plasma [15]. GPx4 has been discovered to be an enzyme protecting biomembranes against oxidative destruction. More recent studies suggest an important role of GPx4 in the regulation of the synthesis of leukotrienes which act as mediators in immune reactions. Moreover GPx4 plays an interesting role with regard to male fertility. During spermatogenesis GPx4 changes from an active enzyme in spermatids into a major structural protein of the mitochondria capsule of mature spermatozoa [16].

In human food selenium is present in two major forms. Feedstuffs derived from animal sources mainly contain selenium in the form of selenocysteine from functional selenoproteins, while selenium from plant derived foodstuffs is present predominantly as selenomethionine. In mineral and trace element supplements selenium is frequently added in the form of the inorganic salts sodium selenite (selenium oxidation state +IV) and sodium selenate +VI) [9]. Selenium from various dietary sources is absorbed by individual mechanisms in the jejunum and in the ileum of mammals. The amino acid derivatives selenomethionine and selenocysteine use the same carriers as their sulphur analogues methionine and cysteine [17]. Selenate uses a sodium- sulphate cotransporter for its absorption, which is driven by the activity of Na+/K+-ATPase at the basolateral enterocyte membrane [18]. Prior to its absorption selenite partially reacts with glutathione and other thiols in the lumen to form selenotrisulfides, which are presumably taken up into the enterocytes by amino acid transporters. Another part of selenite diffuses through the apical membrane and reacts with thiols in the cytosol of enterocytes. The selenium compounds mentioned above are absorbed to a high extent (> 85%) from dietary sources, but differences exist in the absorption time. As a result of the upstream selenotrisulfide synthesis selenite absorption is slower than selenate and selenomethionine absorption [18]. Subsequently the selenocompounds are liberated into the blood stream at the basolateral enterocyte membrane and distributed to the various peripheral tissues. The exact transport mechanism for the various selenium compounds is still not fully understood. Selenomethionine associates with haemoglobin, while selenate and the remaining free selenite was found to be transported with α- and γ-globulins [19, 20, 21]. Thus orally administered selenite presumably enters the peripheral organs in the form of selenotrisulfides or it is reduced in the erythrocytes. Selenate is metabolised during and after its unmodified uptake by the peripheral tissues. This hypothesis of a distinctly different cellular metabolism for selenite and selenate is supported by results of an investigation into intermediary selenium metabolites after intravenous injection of rats with both compounds [22]. Selenite was rapidly taken up by red blood cells, reduced in the erythrocytes to the selenide oxidation state –II and delivered to peripheral organs (liver) in an albumin bound form. In

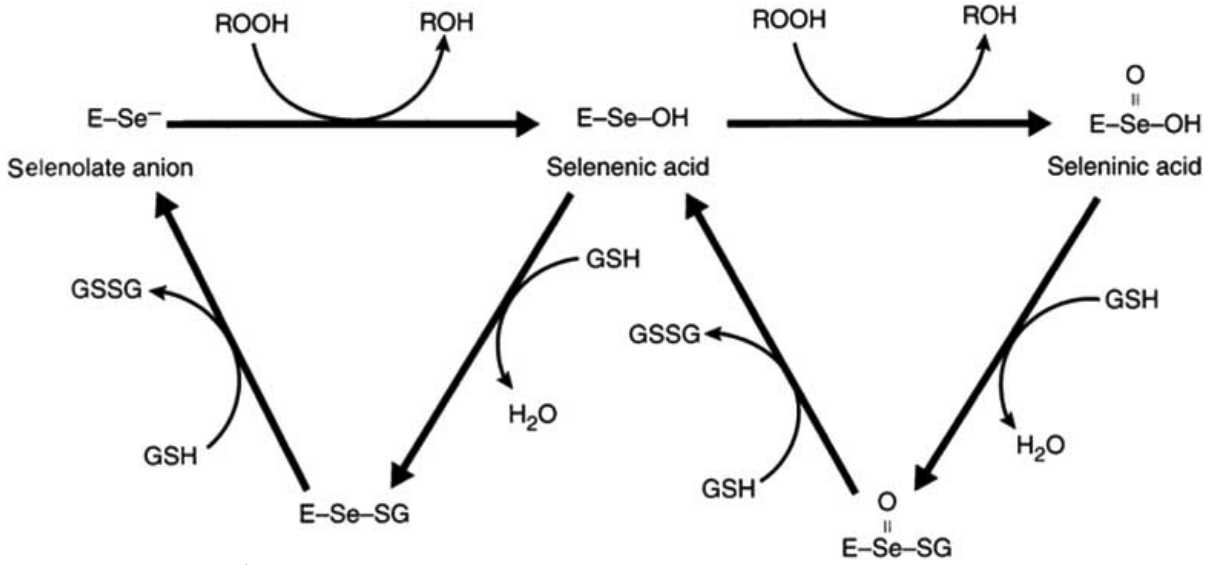


Fig. (1). Reaction mechanism of glutathione peroxidases [13].
At a physiological pH value selenium in glutathione peroxidases is present as a selenolate anion. The reduction of a peroxide effects the oxidation to the selenol oxidation state (selenenic acid). Starting from the selenol oxidation state either the glutathione coupled two step regeneration (reduction) to the selenolate anion or detoxification of a further peroxide and oxidation of the selenol to the oxidation state of seleninic acid can occur.

contrast unmodified selenate could be detected in the bloodstream, and the successive reduction to the oxidation state –II takes place during selenate uptake from plasma to peripheral organs. A fraction of "so-called" acid labile selenium consisting of selenium bound unspecifically to proteins (presumably *via* the formation of Se-S bonds) could be detected. After the intravenous injection with both compounds the main excretion products detected in urine consisted of the methylated forms of selenium [monomethylselenol (MMS) and trimethylselenonium ion (TMS)]. Injection of selenite (Se IV) led to a major peak of these methylated metabolites in urine after 0 – 6 h in comparison to a selenate (Se VI) injection which showed high metabolite concentrations after 6 – 12 hours. Additionally unmodified selenate was excreted after selenate injection.

Selenomethionine is the only selenium compound which can be incorporated unspecifically into proteins instead of its sulphur analogue methionine. The ongoing cellular metabolism of all selenium compounds requires a step by step glutathione-dependent reduction to the selenide oxidation state –II which is the physiological basis for the incorporation of the trace element into the selenocysteine residue of functional selenoproteins by a cotranslational mechanism. Unusually selenocysteine is encoded by the UGA-STOP-codon in the messenger RNA (mRNA) of the functional selenoproteins and synthesised by a cotranslational reaction mechanism starting from a specific serine transfer RNA population (tRNA Ser Sec) and selenium, reduced to the selenide oxidation state (–II) [23, 24, 25]. The regulation of these complex processes is implemented by the SECIS element (selenocysteine incorporating structure) located in the 3´ untranslated mRNA region of functional selenoproteins [26, 27]. Selenium deficiency leads to a down-regulation of the transcript levels of the functional selenoproteins in a tissue and selenoprotein specific manner [28, 29, 30]. Whereas during selenium deficiency the expression of GPx1 and its activity decrease most rapidly the expression level and the activities of GPx4 and GPx2 are altered to a considerably lesser extent. The activity of GPx3 is affected similarly to GPx1 activity during selenium deficiency [31, 32, 33]. Recent molecular biological investigations with rats using the microarray technique have shown that selenium deficiency does not only affect the expression of functional selenoproteins, but also the expression of further genes involved in hepatic xenobiotic metabolism [34, 35]. Microarray technology may provide a helpful tool for the discovery of both positive and critical functions of single nutrients.

2. TYPE 2 DIABETES, INSULIN SIGNALLING AND INSULIN RESISTANCE

Type 2 diabetes is one of the most common metabolic disorders worldwide. In this context peripheral insulin resistance is discussed as an important factor preceding the onset of the disease and promoting its course. "Syndrome X" which includes a cluster of risk factors (hyperglycaemia, hypertriglyceridemia, hyperinsulinaemia) for the development of atherosclerosis and several types of heart diseases is discussed as developing from untreated insulin resistance. The pathophysiology of type 2 diabetes involves impairments of both insulin action and insulin secretion. Insulin sensitivity is determined by the ability of insulin to promote glucose uptake and utilization. Thus, under insulin-resistant conditions, glucose clearance is decreased in response to an insulin challenge [36, 37]. Insulin regulates glucose homeostasis primarily by suppression of hepatic glucose production and stimulation of peripheral glucose uptake [38, 39, 40]. Thus, extensive research on the development of type 2 diabetes has focused on cellular and molecular processes of insulin signalling. In obese rodent models, especially dbdb mice, and in humans the insulin secretion increases with progressive insulin resistance. The relationship is both hyperbolic and tightly coupled. Failure of pancreatic ß-cells to compensate for insulin resistance is critical in the pathogenesis of type 2 diabetes. Factors limiting the ability of ß-cells to respond to an increasing demand remain largely unknown, but probably involve genetic factors as well as glucotoxicity and lipotoxicity. In addition, diminished insulin secretion could be mediated in part by abnormal glucose metabolism. Within the ß-cell glucose metabolism is coupled to insulin biosynthesis and secretion, as well as to ß-cell mass by hypertrophy, hyperplasia, and neogenesis. Moreover, recent studies have shown that the ß-cell itself is an insulin-responsive tissue, demonstrating an additional potential link between peripheral insulin resistance and ß-cell failure [41, 42, 43, 44].

The principal features of insulin action in the organism of healthy mammals at the molecular level have been elucidated during the last two decades (Fig. (**2**)). After binding of insulin to the extracellular α-subunit of its heterotetrameric receptor a conformational change of the protein subsequently activates its intrinsic tyrosine kinase activity. The cytoplasmic kinase domains of the activated receptor transphosphorylate the tyrosines 1158, 1162 and 1163 in the catalytic loop of the kinase domain (β-subunit of the receptor) and subsequently the C-terminal tyrosines 1328 and 1334. The phosphorylation of tyrosine 972 in the juxtamembrane represents the major docking site for downstream interacting proteins. Insulin receptor interacting proteins including the insulin receptor substrates 1 – 6 (IRS 1 – 6) are in turn phosphorylated on tyrosines providing docking sites for SH2 domain containing proteins [46, 47, 48]. The three major pathways emanating from the activated IRS are the PI3K, the CAP-Cbl and the MAPK pathway with the first two pathways mainly involved in the positive control of insulin action [45, 48, 49]. Upon recruitment of IRS1 to the activated IR, IRS1 becomes heavily tyrosine phosphorylated and serves as a large scaffolding protein by binding to several SH2 containing proteins. The most prominent example is the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3-kinase). Binding to p85 recruits the p110 catalytic subunit of PI3-kinase. This interaction positions PI3K in close contact with its substrate phosphatidyl inositol-4,5-bisphosphate (PtdIns-4,5-P_2)-which is then phosphorylated on the 3´-position of the inositol ring yielding the second messenger phosphatidyl inositol-3,4,5-triphosphate (PtdIns-3,4,5-P_3). PtdIns-3,4,5-P_3 recruits the phosphorylation domain-containing protein serine kinases PDK-1, PKB and the atypical PKCλ at the plasma membrane. The activation of this pathway mediates a number of characteristic insulin-induced responses, including translocation of GLUT4 vesicles to the plasma

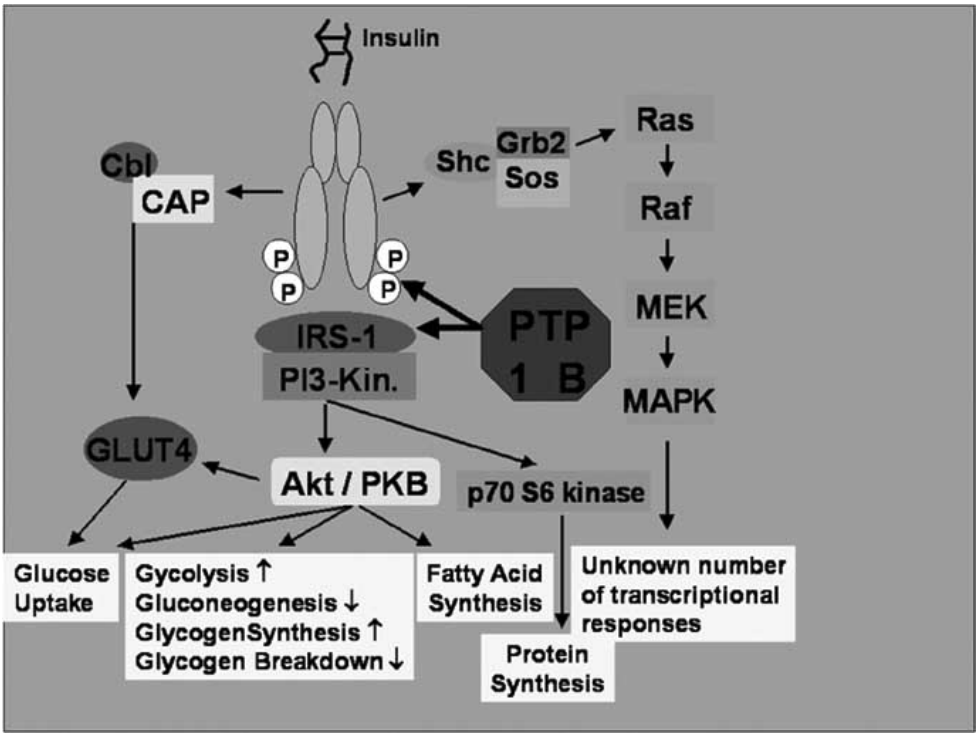


Fig. (2). Major routes of the insulin signalling pathway.

membrane, activation of glycogen synthesis by the protein kinase B mediated phosphorylation of glycogen synthase kinase 3 (GSK3) which regulates glycogen synthase negatively. Furthermore lipogenesis is up-regulated by increasing the expression of the fatty acid synthase gene [50, 51, 52, 53, 54]. Recent investigations suggest that a parallel pathway cooperates with the PI3K pathway to fully induce glucose uptake. This pathway is initiated by the recruitment of the protooncogene Cbl to the activated insulin receptor *via* the adapter protein CAP [55].

Moreover the mitogen activated protein kinase pathway (MAPK) is also induced by insulin *via* Shc and Grb2 association with the insulin receptor as well as IRS molecules triggering an unknown number of transcriptional processes. These processes are partly involved in the growth and differentiation of cells and tissues [56].

Insulin resistance is characterised by a diminished responsiveness to the action of insulin at its multiple target organs. The insulin receptor itself is therefore a primary candidate molecule. Its down-regulation might contribute to the decreased hormone action. Cases of insulin resistance or diabetes have been linked to mutations of the insulin receptor gene [56, 57]. In addition to these rare genetically determined cases, earlier studies showed that the IR tyrosine kinase activity was reduced in insulin-resistant obese mice [58] and in Type 2 diabetic patients [59]. These results were independent of genetic variants of the insulin receptor. A large number of other processes are also discussed as reducing the insulin signal downstream of the receptor during insulin resistance.

Downstream components of the β subunit of the insulin receptor also seem to play a crucial role in the development of insulin resistance. Nearly every signalling protein downstream the insulin receptor was investigated for its involvement in insulin resistance. In obese rodents both a decreased phosphorylation and a reduced protein concentration of insulin receptor substrate 1 (IRS-1) were associated with an increased insulin resistance. Moreover phosphorylation reactions at serine and threonine residues instead of tyrosine residues could be a further cause of a diminished insulin signal transduction. An increased activity and expression of protein kinase c intensifies this mechanism of insulin resistance [60, 61, 62].

Furthermore a connection seems to exist between signalling pathways originally responsible for other physiological processes (e.g. immunological functions, mediation of inflammatory processes, tumour growth and differentiation and apoptosis) and the insulin signalling pathway. In this context it could be demonstrated that the overexpression of Janus kinase in the liver of mice promotes insulin resistance in these animals [63]. The organism of humans and animals also possesses a class of proteins which act as negative regulators of insulin signalling, so-called protein tyrosine phosphatases (PTPs). Their functions include the adjustment of a balanced insulin action and other signalling processes propagated by phosphorylation reactions in cells. In association with type 2 diabetes, obesity and syndrome X an enhanced expression and activity of PTPs is discussed as one possible mechanism for the development and existence of insulin resistance. A mathematical model was also applied which calculates the total insulin action in a cell as the function of concentration, substrate affinity and reaction constant of all known components in the insulin signalling cascade [64]. This theoretical approach allows calculations of the effect of

changes in any component of the insulin signalling pathway, e.g. which effect the increase in PTP activity can have. The large family of protein tyrosine phosphatase proteins can be divided into non-receptor (cytosolic) and receptor PTPs. In particular PTP1B is discussed as acting as an important antagonist of insulin signalling. Therefore the enzyme seems to play a crucial role in the treatment of type 2 diabetes and obesity [64]. Both the specific inhibition of the enzyme by chemical compounds, such as vanadium and the selective reduction of its transcription as well as its translation by antisense oligonucleotides (ASOs) were investigated in order to reduce PTP1B activity and therefore to reduce insulin resistance [65, 66, 67].

The intracellular oxidative and antioxidative balance and especially the concentrations of H_2O_2 and oxidized glutathione (GSSG) possess a pivotal influence on the differentiated regulation of the activity of the enzymes. Stepwise oxidation of the catalytic active Cys215 to sulphenic-, sulphinic- and finally sulphonic acid derivatives by H_2O_2 or formation of glutathionylated intermediates with reduced and/or oxidized glutathione leads to the reversible or irreversible inactivation of the enzyme depending on the oxidation state of the cysteine-sulphur [68, 69, 70].

There are a large number of recent investigations on the influence of cellular antioxidative balance on insulin sensitivity. The conclusions of these investigations are however partially inconsistent.

On the one hand an elevated extra- and intra-cellular glucose concentration as is present in diabetes results in oxidative stress. A large number of cell culture studies demonstrated that the incubation of various cell types with high glucose concentrations (up to 30 mmol/L) increases oxidative stress. The basic mechanism for this increase in oxidative stress is an increased production of superoxide anion radicals generated from glucose autoxidation in the presence of transition metals. Superoxide anion can disproportionate into hydrogen peroxide, which produces the extremely reactive hydroxyl radicals ($^{\cdot}OH$) in the presence of transition metals. Hydrogen peroxide could be demonstrated as reducing insulin signalling directly. Furthermore protein glycation of proteins with a long half life (>10 weeks) leads to the formation of so called ACEs (advanced glycation endproducts) which are able to cause further prooxidative processes and to activate redox sensitive transcription factors like NFkappaB. An increased lipid peroxidation and damage to DNA are further consequences of persistently high glucose concentrations. These results imply that a sufficient supply of antioxidants is necessary to support an efficient therapy for diabetes [71, 72].

On the other hand prooxidative agents of endogenous sources are necessary for an integral function of the insulin signalling pathway and the subsequently triggered metabolic reactions of insulin. Thus insulin per se liberates a burst of hydrogen peroxide in insulin sensitive tissues. This hydrogen peroxide is needed for a coordinated and efficient insulin action [73, 74, 75]. In this context the mechanism is not yet known in detail, but it partly depends on the inhibition of PTPs (antagonists of insulin signalling) by hydrogen peroxide. Furthermore the reduced and oxidized glutathione system seems to play a crucial role for insulin resistance [76, 77].

Two recent investigations in which high activities of glutathione peroxidase and the overexpression of glutathione peroxidase 1 were associated with an increased insulin resistance support this hypothesis and lead to the assumption that a supranutritional supply with antioxidants could cause adverse effects with regard to diabetes [78, 79].

3. THE ANTIDIABETIC ACTION OF HIGH SELENATE DOSES (SELENIUM IN ITS OXIDATION STATE +VI) IN TYPE 1 DIABETIC ANIMALS AND IN TISSUE CULTURES

The Insulinomimetic Action of Trace Elements in the Case of Vanadate

Observations on the antidiabetic properties of trace elements and ultra trace elements were originally made for vanadate. The incubation of hepatocytes with vanadyl sulphate led to an increased glycogen synthesis in these cells [80]. For other cell types (adipocytes, skeletal muscle cells and fibroblasts) positive effects of vanadium compounds on glucose metabolism such as the stimulation of glucose uptake and oxidation and the induction of GLUT1 mRNA could be confirmed [81, 82, 83, 84]. These effects could be attributed to an enhanced phosphorylation of signalling proteins downstream the insulin receptor like protein kinase c (PKC), phosphatidyl inositol-3-kinae (PI3K) and mitogen activated protein kinase (MAPK) [85, 86]. Insulinomimetic properties of vanadate could also be found *in vivo* in type 1- and type 2-diabetic rats and mice when high doses (up to 5 mg per animal and day) of different vanadium compounds (vanadyl, vanadate *bis*[maltolato]oxovanadium) were administered to the animals for several weeks [87, 88, 89, 90]. In more recent investigations it could be demonstrated that the insulinomimetic action of vanadium as the result of an enhanced phosphorylation of certain major proteins of the insulin signalling pathway is caused indirectly by an inhibition of PTPs (including PTP1B) rather than by a direct influence on phosphorylation. Vanadium compounds seem to bind to the catalytic active centre of PTP1B and therefore inhibit the enzyme activity [90, 91].

Effects of High Selenate Concentrations on Glucose Transport and Uptake

In the literature similar findings were made with regard to the insulinomimetic role of selenium or more precisely that of selenate (selenium oxidation state +VI). The first investigation into an insulin-like effect of selenate was made in an experiment with rat adipocytes [92]. Incubation of these cells with 100 µmol/L selenate resulted in a stimulation of glucose transport which was equipotent to that of 1nmol/L insulin. In contrast the incubation of adipocytes with selenite (selenium oxidation state +IV) showed a distinctly lower stimulation of glucose transport. In this study the increase in glucose transport activity by selenate could be attributed to the translocation of the glucose transporters (GLUT-1 and GLUT-2) to the membrane surface. This insulin-like effect of selenate on glucose uptake could also be confirmed in the rat soleus muscle. Incubation of the muscle with either sodium selenite or sodium selenate in increasing concentrations resulted in a markedly stimulated glucose

uptake. A maximum stimulation was reached with a concentration of 100 mmol/L [93].

Antidiabetic effects of selenate could also be observed for its *in vivo* application to type 1 diabetic animals. When selenate was administered to rats and mice with streptozotocin induced type 1 diabetes orally or by intraperitoneal injection for 3 to 8 weeks in daily doses up to half the lethal dose (half lethal dose for selenite and selenate in rats ≈ 3.5 mg/kg body weight) it lowered the elevated blood glucose to nearly the level of non diabetic control animals [94, 95].

The oral treatment of mice with alloxan induced type 1 diabetes with a high dose of selenite (4 mg/kg body weight and day) failed to reduce hyperglycaemia in these animals. This observation is in accordance with our own results (c.v. 4.) and can be interpreted from differences in the intermediary metabolism of selenite and selenate [96].

Insulinomimetic Effects of High Selenate Concentrations on Gene Expression Related to Glucose and Fatty Acid Metabolism

In addition to glucose uptake into insulin sensitive tissues followed by glycolysis and glycogen synthesis insulin fulfils a broad spectrum of other metabolic roles including facilitating the entry of amino acids into cells for the production of cellular protein. Moreover insulin controls the expression of a number of genes. Some insulin responsible genes are key enzymes associated with both carbohydrate and fatty acid metabolism, e.g. glycogen synthase, glucokinase, phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-diphosphatase (F-1,6-Dptase), fatty acid synthase (FAS) and glucose-6-phosphate-dehydrogenase (G6PDH) [51, 53]. Several studies have shown that both vanadate and selenate also possess insulinomimetic properties with regard to glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway. Vanadate was found to inhibit the expression of transfected chimeras of PEPCK in both FTO-2B and H4IIE rat hepatoma cells [96]. Similarly for selenate the oral administration of high doses to type 1 diabetic rats partly normalized the changed expression of glycolytic and gluconeogenic marker enzymes (in diabetes the expression of glycolytic enzymes is down-regulated and gluconeogenic enzymes are up-regulated) to the level of non-diabetic animals. An up-regulation of glycolytic enzymes in particular L-type pyruvate kinase and for gluconeogenesis a down-regulation of PEPCK could be observed. Regulation of the expression of lipogenic enzymes by selenate was also found to be similar to that of insulin. FAS and G6PDH activity were normalized in the livers of type 1 diabetic rats and hepatocytes. Treatment of the diabetic animals or rat hepatocytes in a culture with selenate restored the expression of both FAS and G6PDH, demonstrating that selenate was capable of stimulating lipogenesis in the liver [97, 98, 99, 100].

Further Physiological Effects of High Selenate Doses

A changed lipid metabolism in diabetic humans and animals with syndrome X is assumed to be a factor contributing to a higher risk of heart disease and apoplectic stroke. Against this background a study evaluated cardiac performance in streptozotocin induced type 1 diabetic rats. The treatment of the animals of one group with supranutritional selenate doses improved glucose tolerance in these animals and normalized postprandial plasma glucose levels. Besides high blood glucose concentration the untreated diabetic rats developed increased left ventricular pressure. Treatment with selenate normalized the heart function. Moreover plasma lipid levels, triglycerides, cholesterol and free fatty acids were improved in selenate treated rats. Lowered plasma lipid levels by selenate administration show another potent medical effect of this treatment [95].

Cellular Mechanism Behind the Antidiabetic = "Insulinomimetic" Action of High Selenate Doses

All effects of insulin at the cellular level as described above are initiated by insulin binding to its plasma membrane receptor. Following insulin binding to the α-subunit of the insulin receptor the protein changes its conformation and undergoes a multi-site phosphorylation in the cytosolic β-subunit. By a subsequent phosphorylation of a number of endogenous substrates the insulin signal is spread and amplified by transmission to other signalling proteins. The insulin receptor substrate (IRS) family which includes IRS1, IRS2 and IRS3 (p60) is responsible for a number of insulin effects [102]. In contrast to insulin the insulinomimetics do not bind to the insulin receptor. Nevertheless the results of some studies show an increased phosphorylation of the β-subunit of the insulin receptor and of its substrate IRS1. An increased tyrosine phosphorylation of the insulin receptor's β-subunit has been observed when cells in culture were incubated with vanadate. The insulinomimetic effect of selenate also seems to derive from an enhanced phosphorylation of certain compounds of the insulin signalling pathway. In the above mentioned early study with rat adipocytes not only could a stimulation of glucose transport be attributed to the incubation of these cells with selenate, but also important insight into the mechanism by which selenate develops its antidiabetic properties was given. After incubation of adipocytes the analysis of the whole cell lysate showed an enhanced phosphorylation of several cellular proteins with molecular weights of 170-, 95-, and 60 kDa. Thereby the 170 kDa protein presumably represented IRS1 and the 95 kDa protein was related to the β-subunit of the insulin receptor. In the same study selenite also stimulated glucose transport and therefore intracellular phosphorylation reactions of the insulin signalling pathway. The hypothesis that selenate achieves the increase in phosphorylation reactions by an inhibition of phosphotyrosine phosphatases rather than by an activation of phosphorylation was not supported in this study since selenate showed no inhibitory effect on phosphotyrosine phosphatases in a cell free system. That the opposite seems to be true and that the intermediary selenium metabolism of the intact tissues or cells seems to play a crucial role with regard to the insulinomimetic properties of selenate could be concluded from our recent results (c.v. 4.) [92]. In a study with NIH3T3 HIR 3.5 cells the effect of selenate on IRS1 phosphorylation could be confirmed [103]. In further experiments with 3T3 L1 adipocytes and hepatocytes it could be demonstrated that beside IRS1 and the β-subunit of the insulin receptor the p42 and p44-subunit of MAPK were also affected by an increased

phosphorylation due to incubation with selenate in concentrations up to 1 mmol/L. Studies on general effects of insulin signalling proteins confirmed the the crucial role of PI 3-kinase for stimulation of DNA synthesis, glucose transporter translocation, regulation of glycogen synthase, glycogen synthase kinase-3, the expression of PEPCK and G6PDH expression as well as GLUT-4-mediated glucose transport and membrane ruffling. One protein that has been identified as lying downstream of PI3-kinase is p70 S6 kinase. Both S6 kinase and ribosomal S6 protein play an important role in the initiation of protein synthesis. In a study with primary adipocytes the incubation of these cells with selenate (100 µmol/L – 10 mmol/L) resulted in an increased phosphorylation of S6 kinase. In this study and in the above mentioned early study the insulinomimetic effects of selenate (glucose uptake, increase in the phosphorylation of the β-subunit of the insulin receptor and of S6 kinase) needed a certain incubation time until the onset of the reaction and an even longer period until a maximum response was achieved [104, 105, 106].

On the whole the results of the studies mentioned so far have shown that selenate in high doses (*in vivo*: application of doses up to half the lethal dose = up to 3.5 mg selenium as selenate/kg body weight, *in vitro*: incubation of living cells with 100 µmol/L – 10 mmol/L) has a distinct insulinomimetic property which could be attributed to an increase in the phosphorylation of some major proteins of the insulin signalling pathway.

The most recent investigation regarding the link between selenium and diabetes was carried out in mice in which GPx1, the best characterized selenoprotein was overexpressed. In comparison to control mice with a normal GPx1 expression the overexpression of GPx1 led to a decreased phosphorylation of the β subunit of the insulin receptor and of IRS1 accompanied by an early onset of insulin resistance and obesity [78].

So far *in vivo* investigations have not been carried out on the role of selenite in contrast to selenate with regard to diabetes and there is only little information on the antidiabetic effects of selenium in type 2 diabetic animal models [107].

4. THE ANTIDIABETIC ACTION OF HIGH SELENATE DOSES IN CONTRAST TO SELENITE AND SELENIUM DEFICIENCY IN TYPE 2 DIABETIC MICE AND HEALTHY ANIMALS AND EVIDENCE FOR AN AMBIVALENT ACTION OF SELENIUM WITH REGARD TO DIABETES

Two studies with type 2 diabetic dbdb mice and with healthy growing rats were carried out to investigate the differentiated role of high doses of selenite (selenium oxidation state +IV) and selenate (selenium oxidation state +VI) in type 2 diabetes. In particular the influence of selenium from both compounds applied at the recommended level (0.2 mg/kg diet) and at a moderate supranutritional level (1.0 mg/kg diet) on the expression of diabetes associated genes in healthy animals was examined. Both experiments and their main results are described below.

4.1. Experiment 1 with Type 2 Diabetic dbdb Mice

Twenty-one six week old female dbdb mice (obtained from Harlan Winkelmann, Borchen, Germany) with a mean live weight of 43.7±2.0 g were randomly assigned to 3 groups of 7 animals each (0Se = selenium deficient group, SeIV = selenite-treated group, Se VI = selenate-treated group). All groups received a paletted selenium deficient diet (<0.02 mg Se/kg diet) based on Torula yeast. With the exception of selenium the composition of the diet met the recommendations for mice (4) and was fed for 8 weeks.

The mice of the groups SeIV and SeVI were daily supplemented with sodium selenite and sodium selenate in doses increased weekly, starting with 15% of the LD_{50} for mice and reaching 35% of the LD_{50} in week 8 equivalent to 25-fold the recommended daily intake (LD_{50} selenite and selenate ≈ 3.5 mg/kg body weight) by tube feeding. The animals had ad libitum access to the diet and bidistilled water. Before subjecting the mice to the defined dietary conditions and after 8 weeks of special feeding the insulin sensitivity of the animals was tested. After 8 weeks the mice were decapitated following anaesthetisation with carbon dioxide. The livers were immediately removed, deep frozen in liquid nitrogen and stored at –80°C until further analysis.

4.2. Experiment 2 with Growing Rats

Forty-five growing male albino rats from the strain HK51 weighing 62.8±3.97g were randomly assigned to five experimental groups. The rats were fed a diet for 8 weeks based on Torula yeast composed according to the current recommendations for laboratory rats (NRC 1995) with the exception of selenium. The selenium concentration of the basal diet (group Se0) was below the detection limit of 0.02 mg Se/kg diet. The diets for groups Selenite 0.2, Selenate 0.2 (recommended level), Selenite 1.0 and Selenate 1.0 (supranutritive level) were supplemented with either sodium selenite or sodium selenate in order to obtain final selenium concentrations of 0.2 and 1.0 mg Se/kg diet. The animals had free access to the individual diets and deionised water. After 8 weeks the rats were anaesthetised in a carbon dioxide atmosphere and subsequently decapitated. The livers were excised immediately, deep frozen in liquid nitrogen and stored at -80°C until further analysis.

4.3. Parameters Measured in Both Experiments

- GPx1 activity in liver cytosol from dbdb mice and rats was assayed using the indirect spectrophotometric procedure coupled to glutathione reductase [108].
- Reduced and oxidized glutathione in the liver of dbdb mice and rats was analysed according to the standard protocol coupled to glutathione reductase and DTNB [109]. Sample concentrations were calculated from a standard curve prepared with pure GSSG (concentration range: 0 – 0.066 µmol GSSG/mL).
- The activity of protein tyrosine phosphatases (PTPs) was determined in liver cytosol from dbdb mice and rats according to a modified method which is based on the hydrolysis of paranitrophenyl phosphate (pNPP) [110].

- Additionally the inhibitory effect of different selenium compounds on PTP activity was tested in an *in vitro* system. Liver cytosol was pooled from 3 age-matched adult female dbdb mice fed a standard chow containing 0.25 mg Se/kg diet. PTP inhibition was tested for selenate (oxidation state: +VI), non-enzymatically reduced selenate (using 37% HCl as the reducing agent, oxidation state: +IV), sodium selenite (oxidation state: +IV), selenious acid (oxidation state: +IV) and freshly synthesized selenotrisulfides from the reaction of reduced glutathione and selenite at final selenium concentrations of 25 – 5000 µmol/L. The inhibition of PTPs was expressed as a percent inhibition in comparison with the PTP activity reached in liver cytosol without addition of selenium compounds.
- The expression of PTP1B, the major cytosolic protein tyrosine phosphatase involved in the development and mediation of insulin resistance as well as of glycolytic and gluconeogenic marker enzymes was studied by two step reverse transcriptase PCR (2-step RT-PCR) in the mouse study and by Microarray-Analysis (MWG Rat 10k Array) in the rat study. For both analyses total RNA from liver tissue of dbdb mice and rats was prepared using the acid guanidinium-thiocyanate phenol chloroform extraction [111]. To study gene expression in the dbdb mouse trial by 2-step RT-PCR the extracted RNA was diluted to a final concentration of 2 µg/µL. Three RNA-pools from 2 animals were prepared for each experimental group (0Se, SeIV and SeVI) from the diluted RNA solutions. After reverse transcription of 5 µg (2.5µL) RNA from each pool (RevertAID^{TM} H Minus First Strand cDNA Synthesis Kit, #K1631 from MBI Fermentas) the fragments from the coding sequence of the murine PTP1B, Fructose-1,6-Dptase, PEPCK and GAPDH were amplified by PCR (Table **1**).

After gel electrophoresis in ethidium bromide containing agarose gels the amplification bands were evaluated by measurement of the optical density. Information on gene expression in the rat trial was obtained by the use of Oligoarrays (Rat 10k Array from MWG-Biotech). To compare the effect of the different inorganic Se compounds (selenite and selenate) and concentrations (0.2 and 1.0 mg/kg diet) on gene expression the reverse transcribed RNA extracted from the Se-deficient treatment group was labelled with cyanine 3-dUTP (Cy3) and used as a control sample. Reverse transcribed RNA from the remaining groups was labelled with cyanine 5-dUTP (Cy5). Genes differentially expressed among treatment groups were selected according to the degree of differences in background corrected Cy3/Cy5 hybridisation ratios.

4.4. Results

4.4.1. Glutathione Peroxidase 1

Both in the dbdb mouse study and in the rat study 8 weeks of selenium deficiency led to significantly lower ($p < 0.01$) activities of GPx1 in the liver as compared to selenium supplemented animals (Table **2**).

4.4.2. Glutathione

Thereby the following specific observations could be made: In the dbdb mouse trial the inducing effect of selenate supplementation by daily tube feeding (up to 25-fold the recommended level in week 8) on GPx1 activity was significantly lower ($p < 0.01$) as compared to selenite feeding. In the rat trial the addition of 1.0 mg selenium as selenite or selenate (5-fold the recommended level) led to significantly lower GPx1 activities ($p<0.05$) as compared to rats supplemented according to the recommended dietary level (0.2 mg/kg diet).

Table 1. Oligonucleotide Primers Used for the Amplification of Various Sequences From the Coding Sequence of Murine PTP1B, Fructose-1,6-Dptase, PEPCK and GAPDH Obtained From the Reverse Transcription of mRNA

Examined Gene	Amplified Region From the Coding Sequence	Forward Primer (Tm)	Reverse Primer (Tm)
GPx1	116 - 503	tcattgagaatgtcgcgtct (55.3°C)	tttgagaagttcctggtggg (57.3°C)
PTP1B (mouse)	6 - 706	gatggagaaggagttcgaggag (61.2°C)	acacctgcctcttactgatgg (61.2°C)
PTP1B (rat)	279 - 728	gcacttctgggagatggtgt (59.4°C)	aagaggaaagacccgtcctc (59.4°C)
Fructose-1,6-Dptase	544 - 990	gtcaactgcttcatgctgga (57.3°C)	atgtgcaggagttcctggag (57.3°C)
PEPCK	611 - 1311	agcctttggtcaacaactgg (57.3°C)	atcatctttggtggccgtag (57.3°C)
GAPDH (control)	668 - 971	acgggaagctcactggcatg (61.4°C)	ctacagcaacagggtggtgg (61.4°C)

Table 2. Activity of GPx 1 (mU/mg Protein, Mean±SD) and Concentration of Total, Reduced and Oxidized Glutathione (nmol/mg Protein, M±SD) in the Liver of dbdb Mice and Rats Kept on Selenium Deficiency for 8 Weeks in Comparison to Animals with Supplementation of Selenite- and Selenate

Parameter	Dbdb Mouse Trial				
	0Se	**Se IV**	**Se VI**		
GPx1	171±25.9[a]	703±150[c]	369±43.1[b]		
total glutathione	5.50±0.49[a]	6.69±0.81[b]	6.24±0.97[ab]		
reduced glutathione	2.64±0.32[b]	2.13±0.51[a]	2.37±0.51[ab]		
oxidized glutathione	2.85±0.27[a]	4.56±0.58[b]	3.87±0.49[b]		
% reduced of total	48.03	31.67	37.71		
% oxidized of total	51.97	68.33	62.29		
Parameter	**Rat Trial**				
	0Se	**Selenite 0.2 mgSe/kg**	**Selenate 0.2 mgSe/kg**	**Selenite 1.0 mgSe/kg**	**Selenate 1.0 mgSe/kg**
GPx1	13.4±75.53[a]	1230±227[c]	1148±170[c]	998±73.3[b]	920±140[b]
total glutathione	2.89±0.91[a]	4.39±1.43[ab]	6.25±1.86[b]	5.43±1.24[b]	4.87±1.17[b]
reduced glutathione	2.33±0.61[a]	3.09±1.27[ab]	4.27±1.20[b]	3.52±0.71[b]	3.38±0.92[ab]
oxidized glutathione	0.55±0.35[a]	1.30±0.34[b]	1.98±0.70[b]	1.91±0.58[b]	1.49±0.38[b]
% reduced of total	80.9	70.4	68.3	64.8	69.4
% oxidized of total	19.1	29.6	31.7	35.2	30.6

Significant differences between means (p minimum < 0.05) are indicated by different superscripts within the line.

4.4.2. Glutathione

In both trials selenium supplementation with high supranutritive doses (25-fold the recommended level in the dbdb mouse trial) and at the recommended level (0.2 mg/kg diet) and at a moderately supranutritive level (1.0 mg/kg diet) effected an increase in total liver glutathione concentration (Table **2**). The ratio of oxidized to reduced glutathione also significantly shifted to the oxidized state in both trials.

4.4.3. Protein Tyrosine Phosphatases (PTPs)

In both trials a differentiated effect of selenium supplementation on the activity of cytosolic protein phosphatases in the liver could be observed (Table **3**). In the dbdb mouse study the daily administration of a supranutritive selenate dose effected a significant reduction (inhibition) of PTP activity in comparison to selenite treatment and selenium deficiency. This effect presumably could be attributed to the inhibition of PTPs by Se IV, which is generated during intermediary selenium metabolism. The results of the *in vitro* inhibition test (Fig. (**3**)) indicated that selenate per se did not inhibit PTP activity but that selenium compounds of the oxidation state +IV act as the actual inhibitors of PTP activity. In the rat study both the supplementation of selenite and selenate at the recommended level (0.2 mg/kg diet) and at the supranutritional level (1.0 mg/kg diet) effected a significantly higher PTP activity in comparison to selenium deficiency.

4.4.4. Protein Tyrosine Phosphatase 1B (PTP1B) Expression

Both in the dbdb mouse trial and in the rat trial a significant induction of PTP1B expression (representing the major cytosolic protein tyrosine phosphatase involved in the development and progression of insulin resistance) in the liver was associated with selenium supplementation from both compounds (Fig. (**4**) and Table **4**).

4.4.5. Expression of Gluconeogenic Marker Enzymes (dbdb Mouse Trial and rat Trial) and Expression of Further Genes Associated with Type 2 Diabetes

Differentiated results for both trials were also obtained for the expression of the gluconeogenic marker enzymes F-1,6-DPtase and PEPCK. In the dbdb mouse trial the application of the very high selenate dose significantly reduced the expression of both enzymes in comparison to selenite treated and selenium deficient animals, indicating an amelioration of their diabetes. In the rat trial the expression of both enzymes was higher in all selenium treated groups in comparison to selenium deficiency (Table **5**). Our results further confirmed changes in the expression of genes related to glucose metabolism, insulin resistance and obesity (Table **5**).

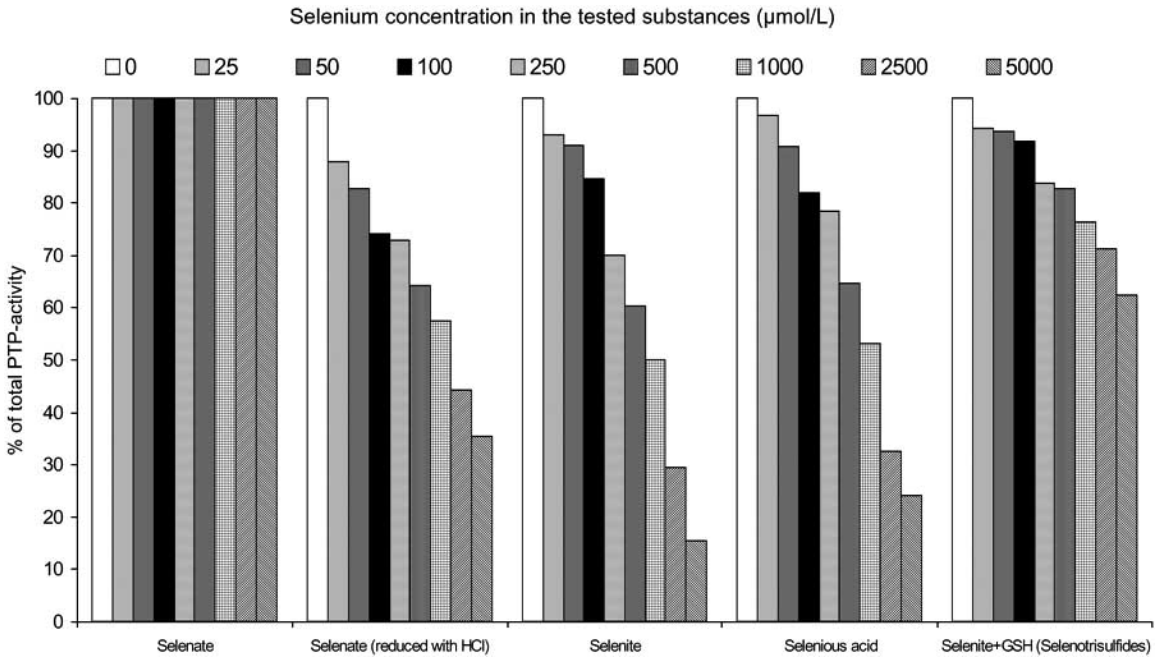


Fig. (3). Inhibition of protein tyrosine phosphatases (PTPs) by different selenium compounds and concentrations.

Table 3. Activity of Protein Tyrosine Phosphatases (U/mg Protein, Mean±SD) in the Liver of dbdb Mice and Rats Kept on Selenium Deficiency for 8 Weeks in Comparison to Animals with Selenite and Selenate Supplementation at Various Concentrations

Dbdb Mouse Trial				
0Se	**Se IV**	**Se VI**		
0.93±0.23[b]	1.20±0.36[b]	0.58±0.15[a]		
Rat Trial				
0Se	**Selenite 0.2 mgSe/kg**	**Selenate 0.2 mgSe/kg**	**Selenite 1.0 mgSe/kg**	**Selenate 1.0 mgSe/kg**
1.99±0.40[a]	2.44±0.44[b]	2.93±0.29[c]	4.27±0.47[e]	3.28±0.38[cd]

Significant differences between means (p minimum < 0.05) are indicated by different superscripts within the line.

4.5. Discussion of our Recent Results and General Discussion

The results from both studies lead to the following hypotheses with regard to the role of selenium in diabetes: As evident from the results of the rat study selenium supplementation at the recommended level (0.2 mg/kg diet) and at a moderately supranutritive level (1.0 mg/kg diet) independent of the selenium compound (selenite or selenate) leads to a high expression and activity of glutathione peroxidase 1 (GPx1). As a consequence of the maximized GPx1 activity the reduced- and oxidized glutathione redox pair shifts to the more oxidized state. The increase in total glutathione concentration and the shift to the more oxidized state presumably triggers an increased glutathionylation of the active site cysteine of PTP1B and a rise in the expression of the enzyme resulting in a higher PTP activity [69]. Experiments with 3T3-L1 adipocytes showed clearly that inhibition of PTP1B activity results in an increased expression of the enzyme [73]. In comparison to the selenium deficient group in our rat study the enhanced PTP activity in selenium supplemented groups may cause a reduction in insulin signalling and the up-regulation of the mRNA of gluconeogenic enzymes and other factors which are increased in type 2 diabetes (Fig. (**6A**)). As is obvious from the dbdb mouse study only selenate supplementation in very high doses seems to evolve antidiabetic properties. Thereby fundamental differences in the metabolism of selenite and selenate play an important role for the antidiabetic effect of selenate *in vivo*.

In contrast to selenite which forms selenotrisulfides during its absorption, selenate is absorbed unmodified and distributed to the tissues in the oxidation state +VI. During and/or after entry into the cells selenate is stepwise reduced

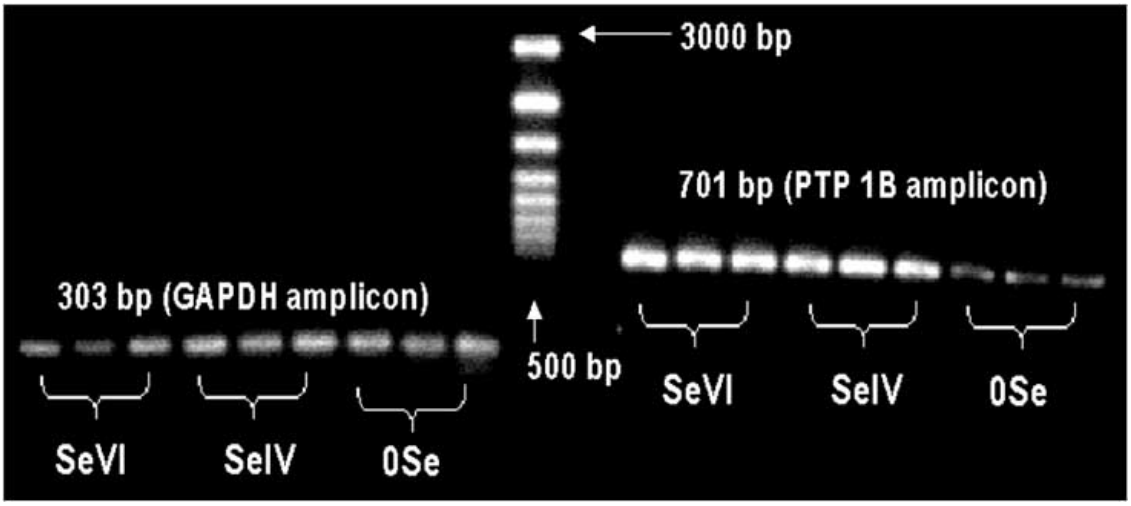


Fig. (4A). Expression of PTP1B in selenium deficient dbdb mice (0Se) compared to dbdb mice after 8 weeks of selenite application (SeIV) or selenate application (SeVI) in relation to their respective GAPDH expression.

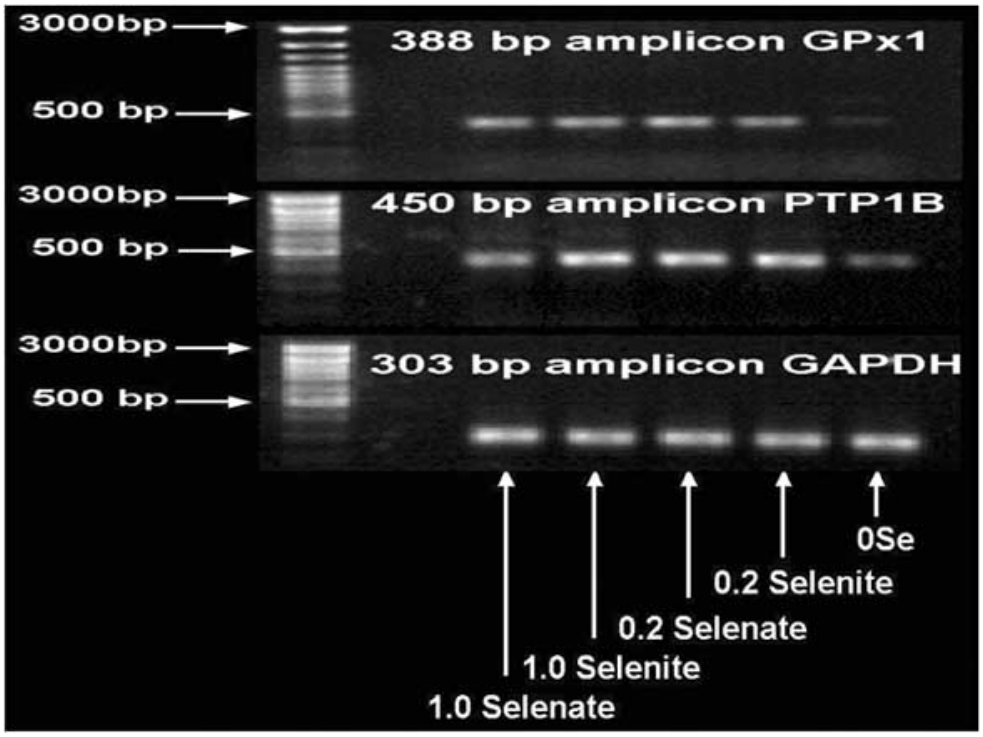


Fig. (4B). Expression of glutathione peroxidase 1 (GPx1) and PTP1B in selenium deficient growing rats (0Se) compared to rats after 8 weeks of selenium supplementation as selenite or selenate in relation to their respective GAPDH expression (Results obtained by 2-step RT-PCR).

Table 4. Expression of PTP1B in Selenium Deficient Growing Rats (0Se, Expression Factor= 1.0) Compared to Rats Receiving Diets Supplemented Either with Sodium Selenite or Sodium Selenate at Two Levels (0.2 mg/kg Diet and 1.0 mg/kg Diet) - Results Obtained by Microarray Analysis (Obtained from Normalised Data of Single Two Channel Microarrays)

	Rat Trial				
	0Se	**Selenite 0.2 mgSe/kg**	**Selenate 0.2 mgSe/kg**	**Selenite 1.0 mgSe/kg**	**Selenate 1.0 mgSe/kg**
Expression against selenium deficiency		1.91	3.81	2.57	5.42

Table 5. Differential Expression of Genes of Functional Selenoproteins, Proteins of Carbohydrate Metabolism and of Phosphatases Associated with Diabetes and Obesity in Selenium Deficient Growing Rats Compared to Rats with Selenium Supplementation Obtained by Microarray Analysis

						Regulation against 0Se = 1.0			
Gene	**0Se**	**Selenite 0.2 mg Se/kg**	**Selenate 0.2 mg Se/kg**	**Selenite 1.0 mg Se/kg**	**Selenate 1.0 mg Se/kg**	**Selenite 0.2**	**Selenate 0.2**	**Selenite 1.0**	**Selenate 1.0**
Functional selenoproteins									
Glutathione peroxidase1	1.76±0.11^{a}	12.31±4.27^{b}	11.67±0.92^{b}	9.65±2.49^{b}	10.36±0.17^{b}	**6.99**	**6.62**	**5.48**	**5.88**
Glutathione peroxidase4	2.71±0.24^{a}	3.60±0.70^{ab}	3.58±0.02^{b}	3.34±0.56^{ab}	3.54±0.02^{b}	**1.33**	**1.32**	**1.24**	**1.31**
Selenoprotein P	78.8±3.39^{a}	162.1±35.3^{b}	166.1±0.77^{b}	134.9±26.0^{b}	153.2±7.71^{b}	**2.06**	**2.11**	**1.71**	**1.94**
Enzymes of glycolysis and glycogen synthesis									
Glucokinase	0.80±0.11^{a}	1.01±0.01^{a}	0.79±0.12^{a}	0.92±0.02^{a}	0.92±0.12^{a}	**1.27**	**0.99**	**1.16**	**1.15**
Pyruvate kinase	1.28±0.11^{b}	0.89±0.28^{ab}	0.54±0.12^{a}	0.78±0.10^{a}	0.97±0.04^{ab}	**0.70**	**0.42**	**0.61**	**0.76**
Glycogen synthase	1.33±0.09^{a}	1.47±0.19^{a}	1.26±0.08^{a}	1.64±0.14^{a}	2.25±0.04^{b}	**1.11**	**0.95**	**1.23**	**1.69**
Enzymes of gluconeogenesis and glycogen breakdown									
Glucose-6-phosphatase	2.26±0.15^{a}	2.54±0.75^{a}	5.01±0.17^{b}	4.55±1.82^{b}	5.15±0.43^{b}	**1.12**	**2.21**	**2.01**	**2.28**
Fructose-1,6-diphosphatase	11.4±0.48^{a}	13.1±3.19^{a}	20.8±1.95^{b}	17.1±4.21^{ab}	15.6±1.13^{b}	**1.15**	**1.82**	**1.50**	**1.37**
Pyruvate carboxylase	1.59±0.09^{a}	2.04±0.46^{b}	2.35±0.15^{b}	2.32±0.40^{b}	2.76±0.16^{b}	**1.28**	**1.48**	**1.45**	**1.73**
Phosphoenolpyruvate carboxykinase	5.73±0.61^{a}	5.82±1.40^{ab}	8.90±0.03^{b}	6.26±2.39^{ab}	9.72±1.54^{b}	**1.01**	**1.55**	**1.09**	**1.70**
Glycogenphosphorylase kinase	0.05±0.05^{a}	0.18±0.09^{b}	0.20±0.01^{b}	0.18±0.05^{b}	0.30±0.03^{b}	**3.25**	**3.60**	**3.36**	**5.48**
Glycogen phosphorylase	0.05±0.01^{a}	0.18±0.12^{ab}	0.16±0.01^{b}	0.14±0.05^{b}	0.21±0.02^{b}	**3.94**	**3.42**	**3.01**	**4.59**
Phosphatases and kinases involved in insulin signalling									
Protein tyrosine phosphatase 1B	0.15±0.06^{a}	0.27±0.17^{ab}	0.47±0.15^{b}	0.24±0.10^{b}	0.38±0.05^{b}	**1.85**	**3.14**	**1.60**	**2.53**
Protein tyrosine phosphatase εc	0.04±0.03^{a}	0.13±0.09^{ab}	0.19±0.06^{b}	0.10±0.03^{b}	0.18±0.01^{b}	**2.90**	**4.19**	**2.07**	**4.09**
Protein phosphatase 2A	0.06±0.03^{a}	0.16±0.09^{ab}	0.33±0.02^{b}	0.27±0.10^{b}	0.26±0.02^{b}	**2.58**	**5.40**	**4.45**	**4.26**
MAP kinase phosphatase	12.2±1.05^{a}	15.2±4.77^{ab}	18.8±1.46^{b}	15.0±1.49^{b}	15.4±0.02^{b}	**1.25**	**1.54**	**1.23**	**1.26**
Serine threonine kinase	46.5±1.48^{a}	59.4±20.4^{a}	96.8±4.33^{b}	71.8±19.3^{b}	62.7±2.15^{b}	**1.28**	**2.08**	**1.54**	**1.35**

Values represent mean±SD of expression of four replicates for each experimental group. Significant differences ($p<0.05$, Students t-test) between means are indicated by different superscripts within a line.

to the oxidation state –II from which selenium can be incorporated into the selenocysteine moiety of the functional selenoproteins (Fig. (**7**)) [17, 18, 19, 20, 22]. The intermediary formation of the oxidation state +IV could thereby play a crucial role for the inhibition of PTP activity as is evident from the results of the *in vitro* inhibition test (Fig. (**3**)).

In the dbdb mouse study the application of both selenite and selenate in very high doses (25-fold the recommended amount) also led to an increase in the expression of PTP1B in comparison to selenium deficiency. But in the case of selenate application in very high doses the inhibitory effect of intermediary selenate metabolites seems to compensate for the increased expression.

Thereby the following changes in the "chain of cause and effect" (Fig. (**6B**)) may happen. Selenate in high doses increases GPx1 activity and shifts the glutathione redox system to a more oxidized state triggering the formation of the glutathionylated (protected) form of PTPs which seems to be the stimulus for the increase in expression. In contrast to the findings for lower selenate doses (rat trial) and selenite treatment (rat trial and dbdb mouse trial) only intermediary selenate metabolites inhibit PTP activity very efficiently when selenate is applied in high doses. Thereby the inhibitory effect seems to compensate for the increased

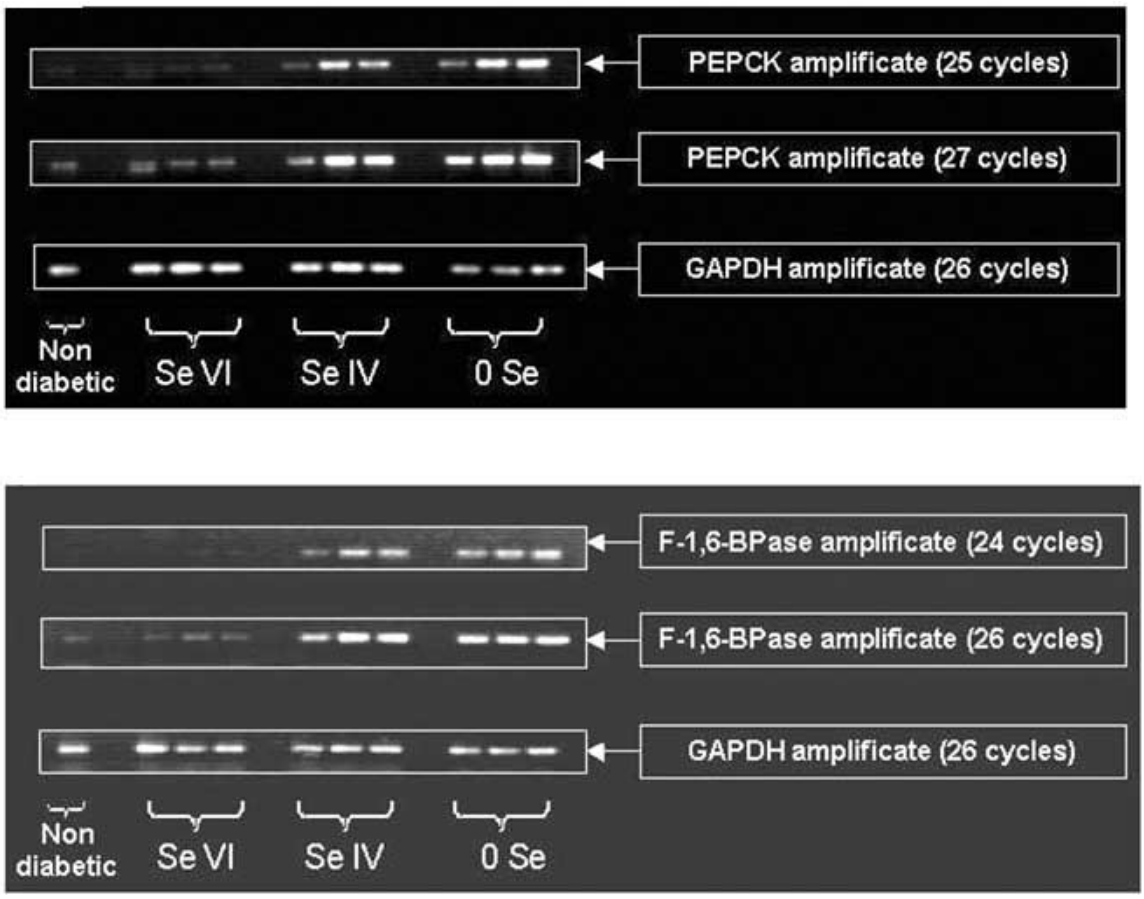


Fig. (5A, B). Expression of Phosphoenol Pyruvate Carboxykinase (PEPCK) [A] and Fructose-1,6-Diphosphatase (F-1,6-Dptase) [B] in selenium deficient dbdb mice (0Se) compared to dbdb mice after 8 weeks of selenite application (SeIV) or selenate application (SeVI) in relation to their respective GAPDH expression.

expression. The reduced (inhibited) PTP activity in turn leads to a correction of insulin resistance and a down-regulation of gluconeogenic marker enzymes.

In both studies one common major result with regard to PTP1B expression could be observed:

Both in selenite and in selenate treated dbdb mice and rats the expression of PTP1B was elevated in comparison to their selenium deficient companions.

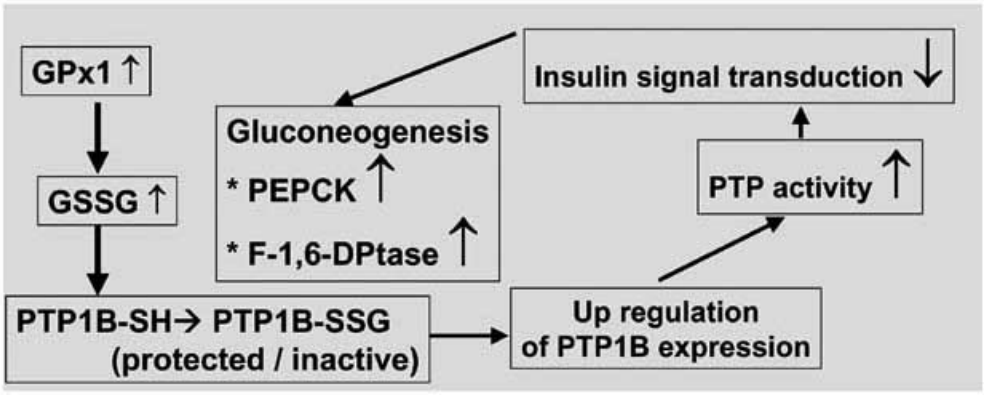
The regulation of PTP1B expression as a negative regulator of insulin signalling is not yet fully understood. On the one hand the reduction of PTP1B expression by treatment with antisense oligonucleotides led to increased insulin signalling [65]. On the other hand an increased insulin sensitivity in obob mice could be observed with leptin treatment despite a concomitant surprisingly up-regulated PTP1B expression [112]. Another study performed with adipocytes reports that the inhibition of PTP1B by hydrogen peroxide led to an increased expression of PTP1B [74].

Due to a high expression and activity of glutathione peroxidase 1 in our studies it seems less likely that hydrogen peroxide was the driving force for the up-regulation of PTP1B expression since this enzyme is involved in the detoxification of hydrogen peroxide. In fact the increase in oxidised glutathione could play an important role triggering an increase in PTP1B expression as evident from the literature [69, 117] and from the results of both studies. Only in the case of administration of very high selenate doses does the inhibitory effect of intermediary selenate metabolites compensate for the increased expression. Future investigations into the precise regulation of PTP1B mRNA expression and PTP1B protein expression should focus on the role of the cellular redox status during these processes.

Furthermore there is evidence for a direct regulation of glycolytic and gluoneogenic enzymes by glutathionylation [118, 119].

Both the data from the dbdb mouse study and from the *in vitro* inhibition assay suggest that the inhibition of PTPs needs high supranutritive doses of selenium. If it is assumed that dbdb mice consume an average of 5 g feed per day, containing the recommended selenium amount for mice (0.2 mg/kg diet, NRC 1985), this would result in a daily uptake of 1 µg selenium. The LD_{50} of selenate and selenite for mice is 3500 µg/kg body weight [113, 114, 115]. Derived from the molecular weight in the case of selenate, selenium accounts for 42% of this amount (1470 µg/kg body weight). In the dbdb mouse trial 15 – 35% of the LD_{50} of selenate were administered to the animals daily. This amounts to an absolute selenium uptake of 10 – 25 µg (10- to 25-fold the

A



B

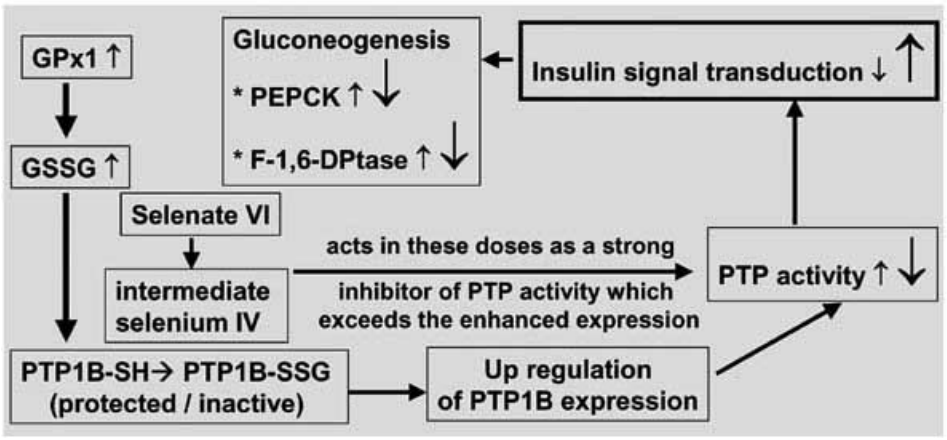


Fig. (6A, B). Chain of cause and effect to explain the ambivalent action of selenium in diabetes.
A: Sufficient selenium supplementation could play a critical role in the development of type 2 diabetes and obesity and needs further investigation.
B: Only extremely high doses of selenate possess insulinomimetic properties due to characteristic changes in the chain of cause and effect.

recommended dietary amount) at an assumed mean body weight of 50 g.

In the *in vitro* inhibition trial the onset of an effective inhibition of PTPs ranged between 50 – 100 µmol/L Se (3.95 to 7.9 µg Se/L). Thus it can be hypothesized that with the oral administration of selenate at regular intervals in the doses used in our present study sufficient selenium concentrations for the inhibition of PTPs were obtained.

The possibility of chronic selenium toxicity cannot be excluded for the doses applied in the dbdb mouse trial. The results from a long-term study on selenium toxicity in rats however suggest that selenium concentrations up to 4 mg/kg diet (20-fold the recommended amount) do not affect animal health [116]. In this study the mortality rate after 2 years (survival rate > 90%) in the group which received 4 mg selenium/kg diet was not higher than in the group fed 1 mg selenium/kg diet. In general information on selenium toxicity is limited. Further investigations into the precise amount of selenium needed for the treatment of diabetes and on the long-term toxicity in different animal species are needed.

5. Conclusions and Perspectives

From the information currently available it is evident that the trace element selenium plays an ambivalent role with regard to diabetes, depending on the chemical form and the applied concentration.

5.1. The One Aspect of Selenium with Regard to Diabetes

Only High Supranutritive Concentrations of Selenate Evolve Antidiabetic Properties

In studies on the antidiabetic effect of selenium which were carried out both in living cells and in type 1- or type 2-diabetic animals an amelioration of the diabetic status by means of a reduced blood glucose concentration and changes in the expression and activity of glycolytic and gluconeogenic marker enzymes could normally be demonstrated as the consequence of selenium supplementation in the form of selenate (selenium oxidation state +VI). Thereby in all experiments high concentrations of selenate were used (animal experiments: daily application of selenate in doses up to half the lethal dose, tissue culture experiments:

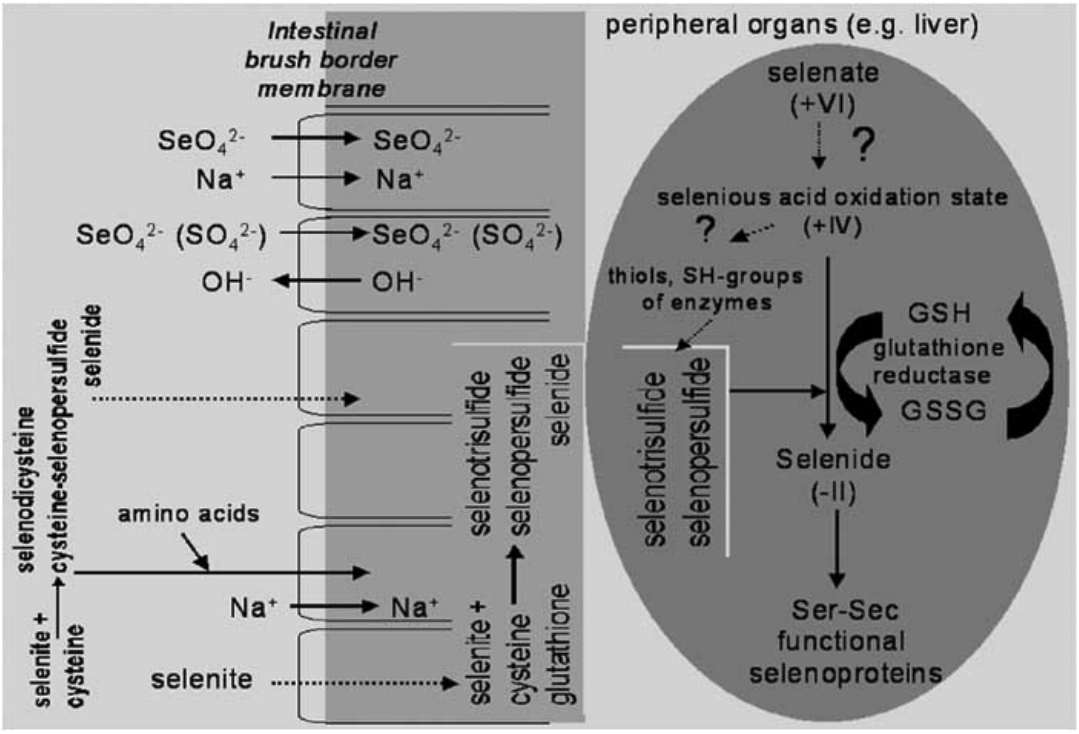


Fig. (7). Selenium absorption and metabolism according to [17, 18, 19, 20, 22].

selenium concentrations in the range of 250 – 5000 µmol/L). In contrast no antidiabetic effect could be obtained by the application of selenite to diabetic animals in comparable concentrations. The insulinomimetic effect of selenate could be attributed to an increase in the phosphorylation of single components of the insulin signalling pathway. In the majority of the studies the increase in phosphorylation was directly measured. From the results of our investigation with dbdb mice and from the *in vitro* inhibition assay with regard to the activity of protein tyrosine phosphatases we concluded that the increase in the phosphorylation is the consequence of protein tyrosine phosphatase inhibition rather than a direct effect of selenate on tyrosine phosphorylation. As is evident from results in cell homogenates and in liver cytosol, selenate (selenium oxidation state: +VI) per se does not inhibit protein tyrosine phosphatase activity. In fact the inhibitory effect of selenate on protein tyrosine phosphatases and with it its insulinomimetic properties seems to derive from the intermediary formation of the oxidation state +IV during and shortly after entering the peripheral tissues [107].

5.2. The Other Aspect of Selenium with Regard to Diabetes

Selenium Supplementation in Moderately Supranutritive Doses Must be Judged Critically with Regard to the Onset and Development of Diabetes and Needs Further Investigation

As recently shown in mice, the overexpression of the best-characterized selenoprotein glutathione peroxidase 1 led to a faster onset of insulin resistance and obesity in comparison with animals in which the enzyme was expressed normally. At the molecular level a decreased phosphorylation of the β subunit of the insulin receptor and of insulin receptor substrate 1 was observed as triggering these effects. The results of this study point in the same direction as our results from the rat study in which an increased expression of PTP1B and an increased activity of general PTP activity could be attributed to selenium supplementation of both selenite and selenate. This increase in PTP expression and activity can also be assumed to weaken the insulin signal and to influence insulin sensitivity and obesity negatively. Enhanced glutathionylation of proteins as the consequence of maximum glutathione peroxidase activity and with it the shift in the glutathione redox pair to the more oxidized state may have an important influence on the differential expression of genes involved in glucose metabolism and genes discussed as relevant factors in the genesis of insulin resistance and obesity [78, 117, 118, 119].

This aspect of selenium, involving a somewhat critical role of the trace element in the context of obesity and insulin resistance needs further intensive investigation.

6. ACKNOWLEDGEMENTS

We thank Prof. Dr. R. Schmidt and Dipl. biol. Sandra Schneider, Biotechnical Centre, Justus Liebig University Giessen, for valuable advice in the RT PCR procedures.

REFERENCES

[1] Römpp Chemie Lexikon 9th edition. Falbe J, Regitz M. (Eds), Volumes 1-6, Georg Thieme Verlag, Stuttgart 1989, 1990, 1991 and 1992.

[2] Combs GF, Combs S (Eds): The role of selenium in nutrition. Academic Press, Orlando 1986; 107-121.

[3] Birringer M, Pilawa S, Flohé L. Trends in selenium biochemistry. Nat Prod Rep 2002; 19: 693-718.

[4] NRC (National Research Council). Nutrient requirements of laboratory animals. 4th revised ed., National Academy Press Washington D.C. 1995.

[5] NRC (National Research Council). Nutrient requirements of poultry. 9th revised ed., National Academy Press Washington D.C. 1994.

[6] NRC (National Research Council). Nutrient requirements of sheep. 6th revised ed., National Academy Press Washington D.C. 1995.

[7] NRC (National Research Council). Nutrient requirements of swine. 10th revised ed., National Academy Press Washington D.C. 1998.

[8] DACH (Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, Schweizerische Vereinigung für Ernährung) Referenzwerte für die Nährstoffzufuhr. 1. Auflage, Umschau Braus GmbH Verlagsgesellschaft, Frankfurt/Main 2000.

[9] NIH (National Institute of Health) (Updated: 08/012004) ods.od.nih.gov/factsheets/selenium.asp.

[10] Brigelius-Flohé R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 2000; 27: 951-965.

[11] Köhrle J. Thyroid hormone deiodinases – a selenoenzyme family acting as gate keepers to thyroid hormone action. Acta Med Austriaca 1996; 23: 17–30.

[12] May JM, Mendiratta S, Hill KE, Burk RF. Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. J Biol Chem 1997; 272: 22607-22610.

[13] Ganther HE, Kraus RJ. Oxidation states of glutathione peroxidase. Methods Enzymol 1984; 107: 593-602.

[14] Brigelius-Flohé R, Mueller C, Menard J, Florian S, Schmehl K, Wingler K. Functions of GI-GPx: lessons from selenium dependent expression and intracellular localization. Biofactors 2001; 14: 101-106.

[15] Takahashi K, Avissar N, Whitin J, Cohen H. Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. Arch Biochem Biophys 1987; 256: 677-686.

[16] Ursini F, Heim S, Kiess M, *et al.* Dual function of the selenoprotein PHGPx during sperm maturation. Science 1999; 285: 1393-1396.

[17] Wolffram S, Berger B, Grenacher B, Scharrer E. Transport of selenoamino acids and their sulfur analogues across the intestinal brush border membrane of pigs. J Nutr 1989; 119: 706-712.

[18] Wolffram S, Arduser F, Scharrer E. *In vivo* intestinal absorption of selenate and selenite by rats. J Nutr 1985; 115: 454-459.

[19] Beilstein MA, Whanger PD. Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine. J Nutr 1986a; 116: 1711-1719.

[20] Beilstein MA, Whanger PD. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. J Nutr 1986b; 116: 1701-1710.

[21] Suzuki KT, Shiobara Y, Itoh M, Omichi M. Selective uptake of selenite by red blood cells. Analyst 1998; 123: 63-67.

[22] Shiobara Y, Ogra Y, Suzuki KT. Speciation of metabolites of selenate in rats by HPLC-ICP-MS. Analyst 1999; 124: 1237-1241.

[23] Sunde RA, Evenson JK. Serine incorporation into the selenocysteine moiety of glutathione peroxidase. J Biol Chem 1997; 262: 933-937.

[24] Böck A, Forchhammer K, Heider J, Leinfelder W, Sawers G, Veprek B, Zinoni F. Selenocysteine : the 21st amino acid. Mol Microbiol 1991; 5: 515-520.

[25] Park SI, Park JM, Chittum HS, Yang ES, Carlson BA, Lee BJ, Hatfield DL. Selenocysteine tRNAs as central components of selenoprotein biosynthesis in eukaryotes. Biomed Environ Sci 1997 ; 10 : 116-124.

[26] Imai H, Sumi D, Hanamoto A, Arai M, Sugiyama A. Molecular cloning and functional expression of cDNA for rat phospholipid hydroperoxide glutathione peroxidase: 3´-untranslated region of the gene is necessary for functional expression. J Biochem Tokyo 1995; 118: 1061-1067.

[27] Walczak R, Hubert N, Carbon P, Krol A. Solution structure of SECIS, the mRNA element required for eukaryotic selenocysteine insertion - interaction studies with the SECIS-binding protein SBP. Biomed Environ Sci 1997 ; 10 : 177-181.

[28] Lei XG, Evenson JK, Thompson KM, Sunde RA. Glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase are differentially regulated in rats by dietary selenium. J Nutr 1995; 125: 1438-1446.

[29] Allan CB, Lacourciere GM, Stadtman TC. Responsiveness of selenoproteins to dietary selenium. Annu Rev Nutr 1999; 19: 1-16.

[30] Wingler K, Bocher M, Flohé L, Kollmus H, Brigelius-Flohé R. mRNA stability and selenocysteine insertion sequence efficiency rank gastrointestinal glutathione peroxidase high in the hierarchy of selenoproteins. Eur J Biochem 1999 ; 259 : 149-157.

[31] Cheng WH, Ho YS, Ross DA, Han Y, Combs GF Jr, Lei XG. Overexpression of cellular glutathione peroxidase does not affect expression of plasma glutathione peroxidase or phospholipid hydroperoxide glutathione peroxidase in mice offered diets adequate or deficient in selenium. J Nutr 1997a; 127: 675-680.

[32] Cheng WH, Ho YS, Ross DA, Valentine BA, Combs GF, Lei XG. Cellular glutathione peroxidase knockout mice express normal levels of selenium-dependent plasma and phospholipid hydroperoxide glutathione peroxidases in various tissues. J Nutr 1997b; 127: 1445-1450.

[33] Lei XG, Dann HM, Ross DA, Cheng WH, Combs GF, Roneker KR. Dietary selenium supplementation is required to support full expression of three selenium-dependent glutathione peroxidases in various tissues of weanling pigs. J. Nutr 1998; 128: 130-135.

[34] Fischer A, Pallauf J, Rimbach G. Selenium and vitamin E dependent gene expression in rats: Analysis of differentially expressed mRNAs. Methods Enzymol 2002; 347: 267-276.

[35] Fischer A, Pallauf J. In: Rimbach, GH, Fuchs J, Packer L. (Eds). Nutrigenomics: Dietary selenium and gene expression. CRC Press, Taylor and Francis 2005, Boca Raton; 441 – 455.

[36] Laaksonen DE, Niskanen L, Lakka HM, Lakka TA, Uusitupa M. Epidemiology and treatment of the metabolic syndrome. Ann Med 2004; 36: 332-346.

[37] Doelle GC. The clinical picture of metabolic syndrome. An update on this complex of conditions and risk factors. Postgrad Med 2004; 116: 30 – 32 and 35-38.

[38] Kodama H, Fujita M, Yamaguchi I. Development of hyperglycemia and insulin resistance in conscious genetically diabetic (C57BL/KsJ-dbdb) mice. Diabetologia 1994; 37: 739-744.

[39] Chua S, Liu MS, Li Q, Yang L, Thassanapaff VT, Fisher P. Differential beta cell responses to hyperglycaemia and insulin resistance in two novel congenic strains of diabetes (FVB-Leprdb) and DBA-Lepob) mice. Diabetologia 2002; 45: 976-990.

[40] Chan TM, Young KM, Hutson NJ, Brumley FT, Exton JH. Hepatic metabolism of genetically diabetic (db/db) mice. I. Carbohydrate metabolism, Am J Physiol 1975; 279: 1702-1712.

[41] Shafrir E, Ziv E, Mosthaf L. Nutritionally induced insulin resistance and receptor defect leading to beta-cell failure in animal models. Ann NY Acad Sci 1999; 892: 223-246.

[42] Kapellen TM, Galler A, Bottner A, Kiess W. Epidemiology, treatment and prevention of type 2 diabetes in children and adolescents. Dtsch Med Wochenschr 2004; 129: 1519-1523.

[43] Bouche C, Shanti S, Kahn R, Glodfine AB. The cellular fate of glucose and its relevance in type 2 diabetes. Endocrine Rev 2004; 25: 807-830.

[44] Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue specific knockout of the insulin receptor in pancreatic b cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 1999; 96: 329-339.

[45] Kido Y, Nakae J, Accili D. Clinical review 125: the insulin receptor and its cellular targets. J Clin Endocrinol Metab 2001; 86: 972-979.

[46] Cai D, Dhe-Paganon S, Melendez PA, Lee J, Shoelson SE. Two new substrates in insulin signaling, IRS5/DOK4 and IRS6/DOK5. J Biol Chem 2003; 278: 25323-25330.

[47] White MF. The insulin signalling system and the IRS proteins. Diabetologia 1997; 40: 2-17.

[48] Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. Biochem J 2000; 346: 561–576.

[49] Baumann CA, Ribon V, Kanzaki M, *et al.* CAP defines a second signalling pathway required for insulin-stimulated glucose transport. Nature 2000; 407: 202–207.

[50] Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995; 378: 785–789.

[51] Girard J, Perdereau D, Foufelle F, Prip-Buus C, Ferre P. Regulation of lipogenic enzyme gene expression by nutrients and hormones. FASEB J 1994; 8: 36–42.

[52] Brazil DP, Hemmings BA. Ten years of protein kinase B signalling: a hard Akt to follow. Trends Biochem Sci 2001; 26: 657–664.

[53] O'Brien RM, Streeper RS, Ayala JE, Stadelmaier BT, Hornbuckle LA Insulin-regulated gene expression. Biochem Soc Trans 2001; 29: 552–558.

[54] Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001; 414: 799–806.

[55] Chiang SH, Baumann CA, Kanzaki M, *et al.* Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. Nature 2001; 410: 944–948.

[56] Pirola L, Johnston AM, van Obberghen E. Modulation of insulin action. Diabetologia 2004; 47: 170-184.

[57] Accili D, Cama A, Barbetti F, Kadowaki H, Kadowaki T, Taylor SI. Insulin resistance due to mutations of the insulin receptor gene: an overview. J Endocrinol Invest 1992; 15: 857–864.

[58] Le Marchand-Brustel Y, Gremeaux T, Ballotti R, Van Obberghen E. Insulin receptor tyrosine kinase is defective in skeletal muscle of insulin-resistant obese mice. Nature 1985; 315: 676–679.

[59] Arner P, Pollare T, Lithell H, Livingston JN. Defective insulin receptor tyrosine kinase in human skeletal muscle in obesity and type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1987; 30: 437–440.

[60] Saad MJ, Araki E, Miralpeix M, Rothenberg PL, White MF, Kahn CR. Regulation of insulin receptor substrate 1 in liver and muscle of animal models of insulin resistance. J Clin Invest 1992; 90: 1839–1849.

[61] Roth RA, Liu F, Chin JE. Biochemical mechanisms of insulin resistance. Horm Res 1994; 41: 51–55.

[62] Qiao LY, Goldberg JL, Russell JC, Sun XJ. Identification of enhanced serine kinase activity in insulin resistance. J Biol Chem 1999; 274: 10625-10632.

[63] Nakatani Y, Kaneto H, Kawamori D, Hatazaki M, Miyatsuka T, Matsuoka TA, Kajimoto Y, Matsuhisa M, Yamasaki Y, Hori M. Modulation of the JNK Pathway in Liver Affects Insulin Resistance Status. J Biol Chem 2004; 279: 45803-45809.

[64] Cheng A, Dube N, Gu F, Tremblay ML. Coordinated action of protein tyrosine phosphatases in insulin signal transduction. Eur J Biochem 2002; 269: 1050-1059.

[65] Asante-Appiah E, Kennedy BP. Protein tyrosine phosphatases: the quest for negative regulators of insulin action. Am J Physiol Endocrinol Metab; 284: E663–E670.

[66] Zinker BA, Rondinone CM, Trevillyan JM, *et al.* PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci USA 2002; 99: 11357–11362.

[67] Harley EA, Levens, N. Protein tyrosine phosphatase 1B inhibitors for the treatment of type 2 diabetes and obesity: recent advances. Curr Opin Investig Drugs 2003; 4: 1179–1189.

[68] Tonks NK. PTP1B: From the sidelines to the front lines! FEBS Lett 2003; 546: 140–148.

[69] Van Montfort RL, Congreve M, Tisi D, Carr R, Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. Nature 2003; 423: 773-777.

[70] Barrett WC, DeGnore JP, Koenig S, *et al.* Regulation of PTP1B *via* glutathionylation of the active site cysteine 215. Biochemistry 1999; 28: 6699–6705.

[71] Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delattre J. Consequences of the diabetic status on the oxidant/antioxidant balance. Diab Metab 2000; 26: 163- 76.

[72] Maritim AC, Sanders RA, Watkins JB. Diabetes, Oxidative Stress, and Antioxidants: A Review. J Biochem Molecul Toxicol 2002; 17: 24-38.

[73] Mahadev K, Wu X, Motoshima H, Goldstein BJ. Integration of multiple downstream signals determines the net effect of insulin on MAP kinase vs. PI 3'-kinase activation: potential role of insulin-stimulated H_2O_2. Cell Signal 2004; 16: 323-331.

[74] Mahadev K, Motoshima H, Wu X, *et al.* The NAD(P)H oxidase homolog NOX 4 modulates insulin-stimulated generation of H_2O_2 and plays an integral role in insulin signal transduction. Mol Cell Biol 2004; 24: 1844-1854.

[75] Mahadev K, Wu X, Zilbering A, Zhu L, Lawrence JT, Goldstein BJ. Hydrogen peroxide generated during cellular insulin stimulation is integral to activation of the distal insulin signaling cascade in 3T3-L1 adipocytes. J Biol Chem 2002; 276: 48662-48669.

[76] Lu B, Ennis D, Lai R, *et al.* Enhanced sensitivity of insulin-resistant adipocytes to vanadate is associated with oxidative stress and decreased reduction of vanadate (+5) to vanadyl (+4). J Biol Chem 2001; 276: 35589-35598.

[77] Khamaisi M, Kavel O, Rosenstock M, *et al.* Effect of inhibition of glutathione synthesis on insulin action: *in vivo* and *in vitro* studies using buthionine sulfoximine. Biochem J 2000; 349: 579-586.

[78] McClung JP, Roneker CA, Mu W, *et al.* Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. Proc Natl Acad Sci USA 2004 101, 8852-8857.

[79] Chen X, Scholl TO, Leskiw MJ, Donaldson MR, Stein TP. Association of glutathione peroxidase activity with insulin resistance and dietary fat intake during normal pregnancy. J Clin Endocrinol Metab 2003; 88: 5963-5968.

[80] Tolman EL, Barris E, Burns M, Pansini A, Partridge R. Effects of vanadium on glucose metabolism *in vitro*. Life Sci 1979; 20: 1159 –1164.

[81] Shechter Y, Karlish SJD. Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (IV) ions. Nature 1988; 284: 556-558.

[82] Dubyak GR, Kleinzeller A. Insulin-mimetic effects of vanadate in isolated rat adipocytes. J Biol Chem 1980; 255: 5306-5312.

[83] Mountjoy KG, Flier JS. Vanadate regulates glucose transporter (GLUT-1) expression in NIH3T3 mouse fibroblasts. Endocrinology 1990; 126: 2778-2787.

[84] Tamura S, Brown TA, Whipple JH, Yamaguchi YF, Dubler RE, Cheng K. A novel mechanism for the insulin-like effect of vanadate on glycogen synthase in the rat adipocytes. J Biol Chem 1984; 259: 6650-6656.

[85] Sekar N, Li J, He Z, Gefel D, Schechter Y. Independent signal-transduction pathways for vanadate and for insulin in the activation of glycogen synthesis and glycogenesis in rat adipocytes. Endocrinology 1999; 140: 1125-1131.

[86] Pugazhenti S, Khandeval RL. Does the insulin-mimetic action of vanadate involve insulin receptor kinase? Mol Cell Biochem 1993; 127: 211-218.

[87] Pederson RA, Ramanadham S, Buchan AMJ, McNeill JH. Long-term effects of vanadyl treatment on streptozotocin-induced diabetic rats. Diabetes 1989; 38: 1390-1395.

[88] Sakurai H, Tschuiya K, Nukatsuka M, Sofue M, Kawada J. Insulin-like effects of vanadyl ion on streptozotocin induced diabetic rats. J Endocrinol 1990; 126: 451-455.

[89] Brichard SM, Bailey CJ, Henquin JC. Marked improvement of glucose homeostasis in diabetic ob/ob mice given oral vanadate. Diabetes 1990; 39: 1326-1332.

[90] Mohammad A, Wang J, McNeill JH. Bis(maltolato)oxovanadium (IV) inhibits the activity of PTP1B in Zucker rat skeletal muscle. Mol Cell Biochem 2002; 229: 125-128.

[91] Schmid AC, Byrne RD, Vilar R, Woscholski R. Bisperoxo-vanadium compounds are potent PTEN inhibitors. FEBS Lett 2004; 566: 35-38.

[92] Ezaki O. The insulin-like effect of selenate in rat adipocytes. J Biol Chem 1990; 262: 6658-6662.

[93] Furnsinn C, Englisch R, Ebner K, Nowotny P, Vogle C, Waldhausl W. Insulin-like *vs*. non-insulin stimulation of glucose metabolism by vanadium tungsten and selenium compounds in rat muscle. Life Sci 1996; 59: 1989-2000.

[94] McNeill JH, Delgatty HL, Battell ML. Insulinlike effects of sodium selenate in streptozotocin-induced diabetic rats. Diabetes 1991; 40: 1675-1678.

[95] Battell ML, Delgatty HL, McNeill JH. Sodium selenate corrects glucose tolerance and heart function in STZ diabetic rats. Mol Cell Biochem 1998; 179: 27-34.

[96] Sheng XQ, Huang KX, Xu HB. New experimental observation on the relationship of selenium and diabetes. Biol Trace Elem Res 2004; 99: 241-253.

[97] Bosch R, Hatzoglou M, Park EA, Hanson RW. Vanadate inhibits the expression of the gene for phoephoenolpyruvate carboxykinase. J Biol Chem 1990; 265: 13677-13682.

[98] Mueller AS, Pallauf J, Rafael J. The chemical form of selenium affects insulinomimetic properties of the trace element: investigations in type II diabetic dbdb mice. J Nutr Biochem 2003; 14: 637-647.

[99] Berg EA, Wu JY, Campbell L, Kagey M, Stapleton SR. Insulin-like effects of vanadate and selenate on the expression of glucose-6-phosphate dehydrogenase and fatty acid synthase in diabetic rats. Biochimie 1995; 77: 919-924.

[100] Stapleton SR, Jivraj S, Wagle A. Insulin and the insulin-mimetic selenium mediate the regulation of glucose-6-phosphate-

dehydrogenase gene expression *via* different signal proteins. FASEB J 1998; 12: 1404.

[101] Becker DJ, Reul B, Ozcelikay AT, Buchet JP, Henquin JC, Brichard SM. Oral selenate improves glucose homeostasis and partly reverses abnormal expression of liver glycolytic and gluconeogenic enzymes in diabetic rats. Diabetologia 1996; 39: 3-11.

[102] Xu P, Jacobs AR, Taylor SI. Interaction of insulin receptor substrate 3 with insulin receptor, insulin receptor-related receptor, insulin-like growth factor-1 receptor, and downstream signaling proteins. J Biol Chem 1999; 274: 15262-70.

[103] Pillay TS, Makgoba MW. Enhancement of EGF and insulin-stimulated tyrosine phosphorylation of endogenous substrates by sodium selenate. FEBS Lett 1992; 308: 38-42.

[104] Stapleton SR, Garlock GL, Foellmi-Adams L, Kletzien RE. Selenium potent stimulator of tyrosyl phosphorylation and activator of MAP kinase. Biochim Biophys Acta 1997; 1355: 259-269.

[105] Okada T, Kawanok Y, Sakakibara T, Hazeki O, Uli M. Essential role of phosphatidyl inositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. J Biol Chem 1994; 269: 3568-3573.

[106] Hei YJ, Farahbakshian S, Chen X, Battel, ML, McNeill JH. Stimulation of MAP kinase and S6 kinase by vanadium and selenium in rat adipocytes. Mol Cell Biochem 1998; 178: 367-375.

[107] Mueller AS, Most E, Pallauf J. Effects of a supranutritional dose of selenate compared to selenite on insulin sensitivity in type II diabetic dbdb mice. J Anim Nutr Anim Physiol 2005; 89: 94 – 104.

[108] Tappel ME, Chaudiere J, Tappel AL. Glutathione peroxidase activities of animal tissues. Comp Biochem Physiol 1982; 73 [B]: 945-949.

[109] Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 1982; 106: 207-212.

[110] Zhu L, Goldstein B. Use of an anaerobic chamber environment for the assay of endogenous cellular protein tyrosine phosphatase activities. Biol Proced Online 2002; 4: 1-9.

[111] Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156-159.

[112] Lam NT, Lewis JT, Cheung AT, *et al.* Leptin Increases Hepatic Insulin Sensitivity and Protein Tyrosine Phosphatase 1B Expression. Mol Endocrinol 2004; 19: [Epub ahead of print].

[113] Hall RH, Laskin S, Frank P, Maynard EA, Hodge HC. Preliminary observations on the toxicity of elemental selenium. AMA Arch Ind Hyg Occup Med 1951; 4: 458-464.

[114] Wilber CG. Toxicology of selenium: a review. Clin Toxicol 1980; 17: 171-230.

[115] Olson OE. Selenium toxicity in animals with emphasis on man. J. Am. Coll Toxicol 1986; 5: 4570.

[116] Jacobs M, Forst C. Toxicological effects of sodium selenite in Sprague-Dawley rats. J Toxicol Environ Health 1981; 8: 575-585.

[117] Klatt P, Lamas S. Regulation of protein function by S-Glutathionylation in response to oxidative and nitrosative stress. Eur J Biochem 2000; 267: 4928-4944.

[118] Hamnell-Pamment Y. Novel methods for the indentification of cellular S-glutathionylated proteins and sites of glutathione dependent modification using affinity chromatography and proteomic analyses. Licentiate Thesis 2005. Institute of Environmental Medicine at the Karolinska Institute, Stockholm, Sweden.

[119] Ziegler DM. Role of reversible oxidation-reduction of enzyme thiols disulfides in metabolic regulation. Ann Rev Biochem 1985; 54: 305-329.

Received: December 31, 2004 Revised: June 15, 2005 Accepted: July 11, 2005

Selenium a risk factor for diabetes development? The regulation of PTP1B may be one part of the puzzle

Andreas S. Mueller [1)], Astrid C. Bosse [1)], Erika Most [1)], Sandra D. Klomann [1)], Sandra Schneider [2)] and Josef Pallauf [1)]

[1)] Interdisciplinary Research Centre
Department of Nutritional Physiology
Justus Liebig University Giessen, Heinrich-Buff-Ring 26 – 32,
D-35392 Giessen (Germany)

[2)] Biotechnical Centre
Justus Liebig University Giessen, Leihgesterner Weg 217,
D-35392 Giessen (Germany)

Correspondence to:
Andreas Mueller
Phone: 0049 641 – 9939230
Fax: 0049 641 - 9939239
Email : andreas.s.mueller@agrar.uni-giessen.de

Keywords: Selenium, Selenite, Selenate, PTP1B, Glutathionylation, Diabetes

Abstract

Despite incontestable antioxidant functions mediated by glutathione peroxidases (GPx) Se has hit the headlines as being involved in the development of obesity and insulin resistant diabetes. Our nutrition physiological study with 7 x 7 growing rats was carried out to investigate mechanisms behind these undesirable effects of Se. One group of rats was fed a Se deficient diet for 8 weeks. The diets of the other 6 groups contained Se as selenite or selenate according to the recommendations (0.20 mg/kg diet) and at two supranutritional levels (1.00 and 2.00 mg/kg diet). A low GPx1 activity in the liver of Se deficient rats corresponded to a low native activity of protein tyrosine phosphatases (PTPs). Augmentation of dietary Se increased GPx1- and PTP-activity. Independent of the effects on PTPs mediated by GPx1 selenate effected a stronger PTP activation compared to selenite. The results for enzymatic PTP analysis could be depicted by Western Blotting with an antibody against protein glutathionylation. In conclusion we assume that supranutritional Se may increase the risk for insulin resistant diabetes by an activation of the insulin antagonistic PTP1B lowering its inhibition through glutathionylation.

1. Introduction

In industrial countries the number of patients suffering from obesity, insulin resistant diabetes and the metabolic syndrome increases permanently [1]. Concomitant in these countries the fortification of foodstuffs derived from plants and animals with vitamins, minerals and trace elements including Se by fertilization and animal nutrition is practised intensified [2 – 5]. Health-conscious individuals frequently consume multivitamin and/or Se supplements in order to optimize their antioxidant defense and to obtain a better protection against several cancer types [6]. Benefits and risks of selenium (Se) supplements for the prevention of type II diabetes are currently subject of a controversial disussion. Antidiabetic effects of Se were reported for animal models and tissue cultures, but they are restricted to very high selenate doses (Se oxidation state +VI), and safety for for humans has not been tested as yet [7, 8]. Oral selenite application however failed to ameliorate diabetes [8, 9]. Opposite effects of Se regarding the development of obesity and insulin resistant diabetes have been shown in transgenic mice overexpressing the peroxide detoxifying selenoprotein GPx1 [10]. Most recent results from two independent U.S. studies with sizable human populations (NHANES III and NPC) have shown independently a distinct correlation between a high Se status and the incidence of type II diabetes [11, 12]. In recent years a number of studies have focussed on the insulin antagonistic protein tyrosine phosphatase 1B (PTP1B) as a molecular target for therapy of obesity and insulin resistant diabetes [13]. In studies with humans [14] and animals it could be shown that PTP1B deficiency, obtained by a lowered expression [15] or enzyme inhibition [8, 16], protects from obesity and insulin resistance whereas high PTP1B activities can accelerate these diseases. In contrast to PTP1B regulation by exogenously applied agents the enzyme underlies a physiological regulation via oxidation of the active site cysteine residue, Cys215 [Fig. 4]. In the presence of H_2O_2 initially a reversibly oxidized sulphenic acid intermediate (PTP1B-SOH) is formed whose further oxidation can be prevented by the formation of a cyclic sulphenyl amide, followed by the reaction with glutathione (GSH/GSSG) to a mixed disulfide with Cys-215, termed glutathionylation. The activity of reversibly oxidized PTP1B and of glutathionylated enzyme can be partially recovered by the addition of dithiothreitol (DTT) or thiol-transferase [17]. The direct reaction

of the reduced Cys-215 SH-group in the presence of high GSSG concentrations (>25 mM) may also lead to glutathionylated PTP1B [18]. Via GPx1 and metabolism of inorganic Se compounds Se influences both intracellular H_2O_2 and GSH/GSSG concentration, the critical metabolites in physiological PTP1B regulation. The aim of our physiological trial with growing rats consequently was to examine if PTP1B may be one part of the puzzle explaining undesirable effects of Se on the development insulin resistant diabetes.

2. Material and methods

2.1 Feeding trial with healthy growing rats

49 healthy growing male albino rats from the institutes own strain HK51 were randomly assigned to 7 experimental groups of 7 animals each. The Se deficient basal diet (group 0Se; <0.02 mg Se/kg diet) was based on Torula yeast (30% Torula yeast, 5% cellulose, 5% glucose, 5% sucrose, 5% soybean oil, 0.6% DL methionine, 0.05% tryptophan, 3.5% mineral premix, 1.0% vitamin premix 0.2% cholinechloride 44.65% maize starch) and composed according to the recommendations for laboratory rats [19]. The diets for groups 0.2 Selenite, 0.2 Selenate (recommended dietary level), 1.0 Selenite, 1.0 Selenate, 2.0 Selenite and 2.0 Selenate (supranutritional levels) were supplemented with either sodium selenite or sodium selenate to obtain final Se concentrations of 0.2, 1.0, and 2.0 mg Se/kg diet. The animals were kept individually and had ad libitum access to the diet and bidistilled water. After 8 weeks the rats were decapitated under anaesthesia and livers were excised and stored at –80°C until further analysis. The protocol of the animal study was approved by the regional council of Giessen.

2.2 Determination of Se concentration

Se concentration in the diets, the livers and plasma was determined by hydride generation atomic absorption spectrometry (HGAAS) as reported previously [20]. Certified samples from the “National Institute of Standard and Technology” (soft winter flour, NIST No. 8438), (bovine liver, NIST No. 1577 b), and from “Medichem” (control serum, Metalle S) served as reference material for Se determination in the different matrices.

2.3 Liver GPx1 and plasma GPx3

Glutathione peroxidases 1 and 3 were measured in the 10,000 x g cytosolic supernatant of 1:10 (w/v) liver homogenates or undiluted plasma by the indirect spectrophotometric procedure coupled to glutathione reductase and NADPH consumption [21]. One unit of GPx1 or 3 activity was defined as one micromole NADPH oxidized per minute and normalized to 1 mg protein.

2.4 Total glutathione and oxidized glutathione

The concentration of total glutathione (GSH + reduced GSSG) and oxidized glutathione (GSSG) in rat liver was analyzed in the 10,000 x g cytosolic supernatant according to the standard protocol coupled to glutathione reductase and DTNB [22]. Sample concentrations were calculated from a standard curve prepared with pure GSSG (concentration range: 0 – 0.066 µmol GSSG/mL).

2.5 Liver protein tyrosine phosphatase activity (PTP) under native and reducing conditions

Differentiated measurement of (PTPs) was carried out using a modified protocol basing on paranitrophenyl phosphate (pNPP) hydrolysis [23, 24].

Step 1: For the analysis of PTP activity 1:5 (w/v) liver homogenates were prepared under nitrogen gassing in a non-reducing HEPES buffer [50 mmol/L 4-(-2-Hydroxyethylpiperazine-1-ethanesulphonic acid (HEPES), 50 mmol/L NaCl, 1 mmol/L EDTA and 0.1 mmol/L Phenylmethylsulphonylfluoride (PMSF) pH = 7.4]. Cytosol was obtained by centrifugation at 10,000 x g for 30 min at 2°C and brought to a final dilution of 1:25 (w/v).

Step2: 10 µL of diluted liver cytosol were preincubated at 25°C in 240 µL of the DTT-free HEPES buffer for 3 minutes. Then 250 µL HEPES-buffer containing 20 mmol/L pNPP were added and further incubated for 5 min. The reaction was terminated by the addition of 500 µL 2M NaOH and absorption was read in a spectrophotometer (Beckmann DU 50) at 410 nm. A blank without cytosol was carried along. Native PTP activity was calculated using an extinction coefficient of 0.0166 μM^{-1} x cm^{-1} for the paranitrophenolate ion and normalized

to 1 mg protein. To determine the percentage of glutathionylation, reversible by DTT, enzymatic measurement was repeated as described, but HEPES buffer containing 2.5 mmol/L DTT was used.

2.6 Western Blot analysis of PTP1B-glutathionylation

For analysis of PTP1B glutathionylation 1:10 (w/v) liver homogenates were prepared in a non-reducing RIPA lysis buffer [50 mmol/L TRIS-HCl, 150 mmol/L NaCl, 1 mM Phenylmethylsulphonylfluoride (PMSF), 1 mM EDTA, 1.0 % sodium desoxycholate, 0.1 % sodiumdodecylsulphate (SDS) and 1% TritonX-100, pH = 7.4]. After centrifugation (10,000 x g, 30 min, 2°C) the cytosol was diluted to 1:50 (w/v). 40 µg of protein were separated according to the standard method [25] but under non-reducing conditions on 15% SDS-polyacrylamide gels (50 mA, 4°C, 2h). Separated proteins were transferred onto a PVDF membrane (PALL Biotrace 0.45 µm^{TM}) by semi-dry blotting [25 min at constant 6V (~ 60 mA)]. After blocking membranes overnight at 4°C in TBST (20 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.1% Tween 20, pH = 7.6) containing 5% non-fat dry milk and 0.2% bovine serum albumine (BSA) analysis was continued by a 2h incubation with the Anti Glutathione Antibody (Virogen 101-A-100) in TBS (1:1500) buffer and a 1h incubation with the secondary antibody (1:3000) linked to alkaline phosphatase (Goat Anti-Mouse IgG-h+l). Membranes were stained in reaction buffer (0.1 mol/L TRIS, 0.1 mol/L NaCl, 0.05 mol/L $MgCl_2$) containing 0.00375% Nitro-Blue Tetrazolium (NBT)-and 0.0025% 5-bromo-4-chloro-3-indoylphosphate (BCIP). Optical density of the ~50 kDA PTP1B-band was evaluated (Gene Tools, Syngene) on scanned membranes (CanoScan LiDe 500F). To examine the in vitro effect of selenite and selenate on PTP1B glutathionylation a pooled liver sample from the Se deficient group was homogenized 1:5 (w/v) in TRIS-HCl buffer [50 mmol/L TRIS-HCl, 150 mmol/L NaCl, 1 mM Phenylmethylsulphonylfluoride (PMSF) 1 mM EDTA, pH = 7.4] and cytosol was prepared as described above. 50 µL of cytosol were incubated with 50 µL of selenite or selenate solutions obtaining final Se concentrations of 25, 50, 75 and 100 µmol/L for 10 min. Then 400 µL of the RIPA buffer were added [final dilution 1:50 (w/v)] and an aliquot was subjected to Western Blot analysis as decribed.

2.7 Protein content

The protein content of liver cytosol, including samples for Western Blotting, was determined using a standard method [26].

2.8 GPx1 and PTP1B mRNA expression

RNA isolation was carried out using the acid guanidine thiocyanate phenol chloroform method [27]. Reverse transcription (RT) of RNA followed by PCR reactions for the examination of GPx1 and PTP1B expression in the liver was carried out as described detailed previously [8]. Using 2.5 µg of cDNA (obtained from reverse transcribed RNA) the PCR reactions for the amplification of specific fragments within the open reading frame (ORF) of GPx1, PTP1B and β Actin (control) were carried out in a reaction volume of 50 µL with a standard program for the single cycles.

Gene (Gene bank accession number)	**Amplified region within the ORF**	**Forward Primer (Tm)**	**Reverse Primer (Tm)**
Rat GPx1 (NM 030826_1)	116 – 503 (396 bp)	tcattgagaatgtcgcgtct (55.3°C)	tttgagaagttcctggtggg (57.3°C)
Rat PTP1B (NM 012637_1)	279 – 728 (449)	gcacttctgggagatggtgt (59.4°C)	aagaggaaagacccgtcctc (59.4°C)
Rat β Actin (NM 031144_1)	301 – 696 (395)	tgttaccaactgggacgaca (59.4°C)	tctcagctgtggtggtgaag (59.4°C)

The standard program was as follows: Initial denaturation: (95°C: 3 min) 1x ; Amplification cycles: [Denaturation: 95°C: 45 sec, Annealing: primer specific temperature: 40 sec, Extension: 72°C: 55 sec] x-times, Final Extension: (72°C: 5 min) 1x.

2.9 Statistical Analysis

A one way analysis of variance (ANOVA) was performed using “SPSS 14.0 for Windows”. If homogeneity of variance was given the LSD-test was used to examine differences of means, if not the Games Howell test was utilized. Values in the tables are given as M±SD (n=7 animals per group) and include 3 repetitions per parameter. Error probabilities and tests used are indicated in the table-legends. Correlation- and regression analyses were also performed with “SPSS 14.0 for Windows”.

3. Results

3.1 Zootechnical parameters

The Se concentration of the Se deficient basal diet was below our detection limit of 20.0 µg Se/kg. The analysed dietary Se concentrations (µg/kg) for the Se supplemented groups were in accordance with the amounts scheduled in the experimental design: 0.2 Selenite: 201±11.7; 0.2 Selenate: 187±9.80; 1.0 Selenite: 934±71.4; 1.0 Selenate: 961±25.4; 2.0 Selenite: 1932±69.7; 2.0 Selenate: 1904±77.4.

Initial body weight in all experimental groups was not different (61.0±2.96). At the end of the trial mean body weights of rats from all Se supplemented groups (0.2 Selenite: 352.3±20.9; 0.2 Selenate: 357.0±22.0; 1.0 Selenite: 336.6±9.83; 1.0 Selenate: 330.9±13.1; 2.0 Selenite: 327.5±17.1; 2.0 Selenate: 326.6±13.3) were significantly higher compared to group 0Se (307.0±11.3). Feed intake in group 0Se was lowest within the experimental groups (992.8±49.6) and comparably as high as in the groups supplemented with 2.0 mg Se/kg diet (2.0 Selenite: 1013±27.0; 2.0 Selenate: 993.0±43.0). Rats supplemented at the recommended level had a significantly higher feed intake compared to the above mentioned groups (0.2 Selenite: 1080±61.8; 0.2 Selenate: 1084±55.7). Feed intake in the groups supplemented with 1.0 mg Se/kg diet (1.0 Selenite: 1024±26.5: 1.0 Selenate: 1031±54.2) ranged between the groups supplemented with 0.2 and 2.0 mg Se/kg.

3.2 Se status and Se- and glutathione dependent redox system in liver and plasma

Expression of GPx1 mRNA in the liver of selenite or selenate supplemented rats at all three levels was 8 to 10-fold higher compared to Se deficient rats (Fig.1).

Liver Se concentration clearly indicated the Se depletion in group 0Se (Table 1). Se supply led to a dose dependent increase in liver Se content. Thus in the groups supplemented with 0.2, 1.0, and 2.0 mg Se/kg diet 65,-125,-and 167-fold higher Se concentrations could be measured compared to group 0Se. GPx1 activity in group 0Se reached only 1.10 to 1.27% of the activities measured in the Se supplemented groups (Table 1).

Data indicated that a plateau in GPx1 activity was already achieved with 0.2 mgSe/kg diet, and additional Se supply did neither produce an increase in liver GPx1 nor was there any negative influence on GPx1 activity. Both total glutathione and the portion of oxidized glutathione were significantly higher in all Se supplemented groups compared to group 0Se, with the highest values for total glutathione in groups 2.0 Selenite and 2.0 Selenate (Table 1). Se deprivation was also reflected by plasma Se concentration in group 0Se. Augmentation of dietary Se supply to the recommended level and to supranutritional concentrations led to a dose dependent increase in plasma Se which was however not as distinctive as analyzed in the livers (Table 1). GPx3 activity in group 0Se reached only 1.11 to 1.50% compared to the Se supplemented groups (Table 1). As found for liver GPx1, Se supplementation to supranutritional levels (1.0 and 2.0 mg Se/kg diet) did neither increase GPx3 activity nor show a negative influence on the enzyme´s activity.

Table 1: Se status and Se- and glutathione dependent redox system in the liver and plasma of rats fed diets with different selenite or selenate amounts compared to Se deficient companions

Group Parameter	0 Se	0.2 Selenite	0.2 Selenate	1.0 Selenite	1.0 Selenate	2.0 Selenite	2.0 Selenate
Liver							
Se conc. (µg/kg FM)	18.4±2.0 (a)	1054±73 (b)	1343±104 (c)	2469±123 (d)	2292±130 (d)	3010±113 (e)	3060±180 (e)
GPx1 (mU/mg prot.)	9.96±6.21 (a)	851±93.1 (c)	909±184 (bc)	910±87.5 (c)	781±81.1 (bc)	612±86.2 (bc)	905±134 (c)
Total GSH (nmol/mg prot.)	27.9±4.11 (a)	34.5±5.12 (b)	40.1±4.94 (bc)	42.0±5.78 (bc)	37.8±4.51 (b)	45.1±5.25 (c)	47.9±5.06 (c)
Oxidized GSSG (nmol/mg prot.)	1.70±0.26 (a)	13.5±1.66 (b)	11.8±3.21 (b)	14.1±2.17 (b)	12.6±2.04 (b)	15.6±2.35 (b)	14.9±4.40 (b)
% oxidized of total glutathione	6.15±0.82 (a)	39.4±2.55 (c)	29.7±8.66 (bc)	33.8±3.93 (bc)	33.9±5.94 (bc)	34.6±2.02 (b)	31.7±9.84 (bc)
Plasma							
Se conc. (µg/L)	22.9±3.10 (a)	544±20.1 (b)	571±31.8 (b)	648±21.4 (c)	610±44.4 (bc)	706±30.8 (d)	707±37.8 (d)
GPx3 (mU/mg prot.)	1.80±0.85 (a)	119±10.9 (b)	149±22.6 (bc)	161±24.8 (c)	133±14.1 (bc)	137±15.9 (bc)	141±18.0 (bc)

Liver Se ($p<0.001$, LSD-test); GPx1 ($p<0.001$, LSD-test); Total glutathione, Oxidized glutathione ($p<0.05$, LSD-test); % oxidized of total glutathione ($p<0.01$, Games-Howell-test); Plasma Se ($p<0.001$, LSD-test); GPx3 ($p<0.001$, LSD-test)

3.3 Regulation of liver PTP1B

The expression of PTP1B mRNA was 2.5 to 3.5-fold reduced in group 0Se compared to rats with Se supplementation as selenite or selenate at all dietary levels examined (Fig.1).

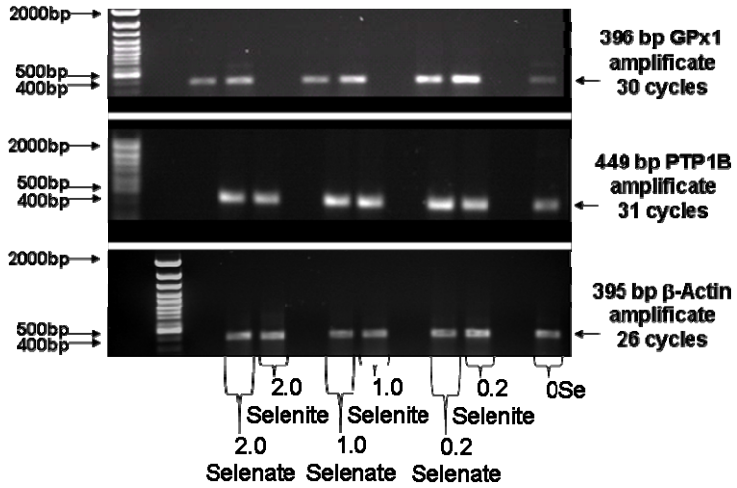


Fig. 1. mRNA expression of GPx1 and PTP1B in the liver of rats fed diets with different selenite or selenate amounts compared to Se deficient companions

Native liver PTP activity measured without DTT was 1.48 to 3.68-fold higher in rats fed Se supplemented diets compared to their Se deficient littermates (Table 2). The lowest activity difference in comparison to goup 0Se was achieved in group 0.2 Selenite. From group 0.2 Selenate onwards the difference in PTP activity compared to group 0Se was distinctly higher. The highest native PTP activity was reached in group 2.0 Selenate. PTP measurement with DTT addition increased PTP activity in all groups, indicating the regeneration of PTP enzyme inhibited by reversible glutathionylation. PTP activity measured with DTT addition was still the lowest in group 0Se compared to all groups with Se supply, but the factors for activity difference were diminished and ranged only from 1.18 to 1.37-fold. The remaining difference in PTP activity can be explained by a higher expression due to Se supplementation (Fig. 1). The highest percentage of glutathionylation and therefore inactivation of PTPs was measured in group 0Se (Table 2). An increase in dietary Se concentration led to a dose-dependent loss of PTP glutathionylation. A comparison of the groups supplemented with selenite and selenate at the same dietary level (0.2, 1.0, and 2.0) revealed a significantly lower PTP glutathionylation for selenate supplementation in each case (Table 2).

Table 2: PTP activity under native and reducing conditions and calculated ratio of PTP glutathionylation in the liver of rats fed diets with different selenite or selenate amounts compared to Se deficient companions

Group Parameter	0 Se	0.2 Selenite	0.2 Selenate	1.0 Selenite	1.0 Selenate	2.0 Selenite	2.0 Selenate
PTP activity -native- (U/mg prot.)	0.59±0.13 (a)	0.88±0.16 (b)	1.22±0.18 (cd)	1.15±0.09 (c)	1.41±0.18 (d)	1.66±0.14 (e)	2.18±0.29 (f)
PTP activity -2.5 mM DTT- (U/mg prot.)	1.78±0.08 (a)	2.23±0.21 (bc)	2.21±0.35 (ac)	2.17±0.16 (bc)	2.10±0.26 (ac)	2.34±0.10 (bc)	2.45±0.24 (bc)
PTP glutathionylation (%)	66.7±7.89 (a)	60.7±4.73 (a)	44.4±4.87 (bcd)	46.8±2.23 (bd)	33.27±7.91 (cef)	29.0±4.67 (eg)	10.2±13.9 (fg)

Significant differences within a line are indicated by different small letters (n=7 animals per group) PTP activity native ($p<0.01$, LSD-test); PTP activity -2.5 mM DTT- ($p<0.01$, LSD-test); PTP glutathionylation in % ($p<0.05$, Games-Howell-test)

The coherence between liver and plasma Se concentration (resulting from dietary Se supplementation at different levels) and the Se compound used (selenite or selenate) and PTP activity as well as PTP glutathionylation was pointed out by correlation- and regression-analyses (Fig. 2A-D). A highly positive correlation between liver Se concentration and native PTP activity could be demonstrated for selenite ($r = 0.88$; $p<0.001$) and selenate ($r = 0.91$; $p<0.001$), whereas the correlation between liver Se concentration and glutathionylation was strongly inverse for both Se compounds (selenite: $r = -0.87$; $p<0.001$; selenate: $r = -0.88$; $p< 0.001$). Slopes of linear regression indicated a faster rise in native PTP activity and a more distinct loss of PTP glutathionylation due to selenate supply compared to selenite supply (Fig. 2A, B). These results are in accordance with enzymatic PTP measurement where selenate fed rats had a higher native PTP activity and a lower PTP glutathionylation compared to selenite fed rats (Table 2). By correlation analyses and square regression comparable coherences could be also shown for the relations between plasma Se concentration and liver PTP activity- and glutathionylation (Fig. 2C, D).

The results obtained by measurement of PTP activity could be visualized by Western Blot analysis using an antibody detecting “Protein Glutathionylation”. PTP1B glutathionylation was 1.5 to 3.5-fold lower in Se supplemented rats compared to their littermates of group 0Se [Fig.

3A]. An increase in dietary Se supply led to a decrease in PTP1B glutathionylation. As found for enzymatic PTP measurement Western Blotting of liver cytosol from rats fed selenite versus rats fed selenate at the same dietary level (0.2, 1.0 and 2.0) revealed a lower PTP1B glutathionylation for animals receiving selenate [Fig. 3B].

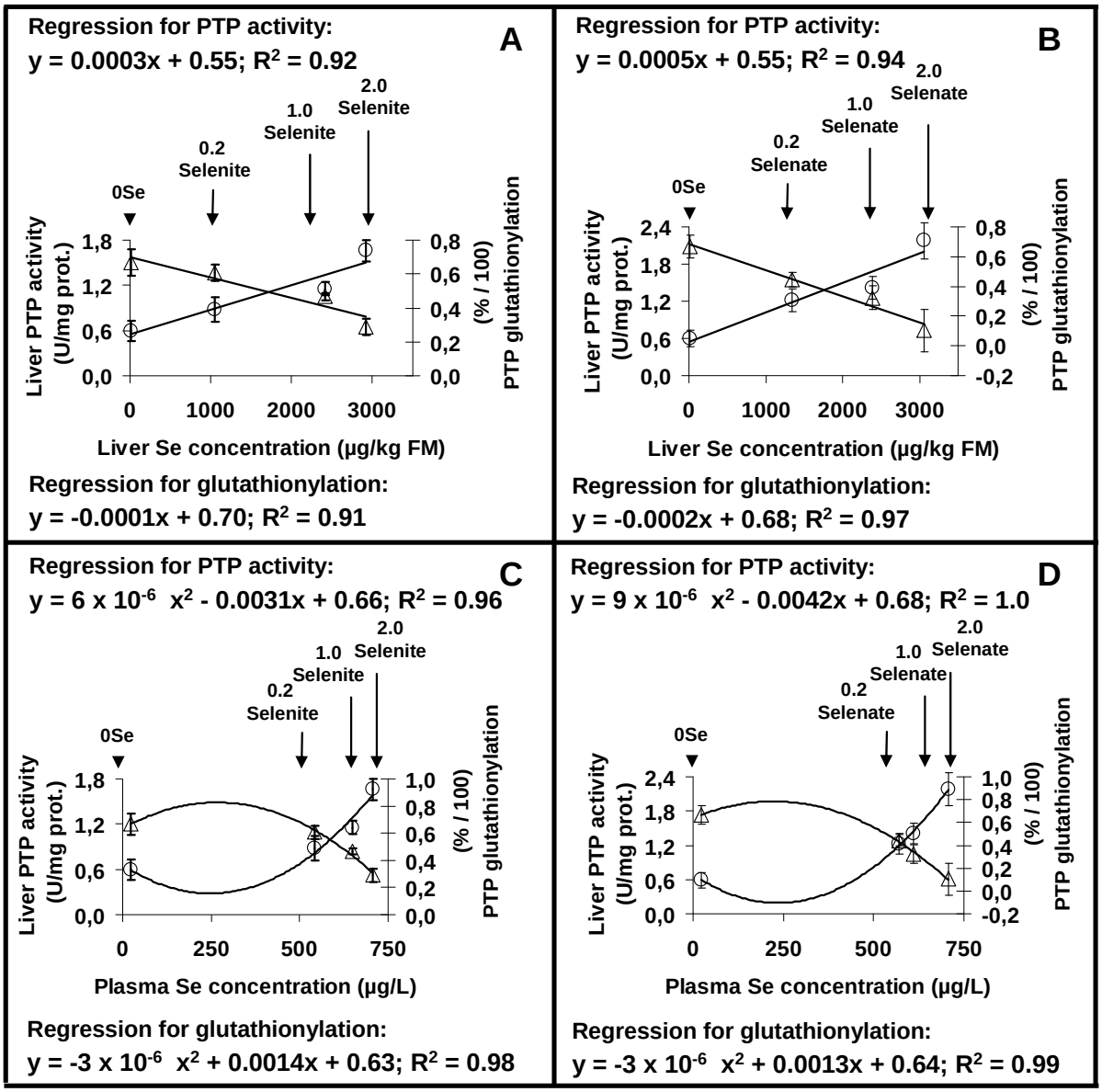
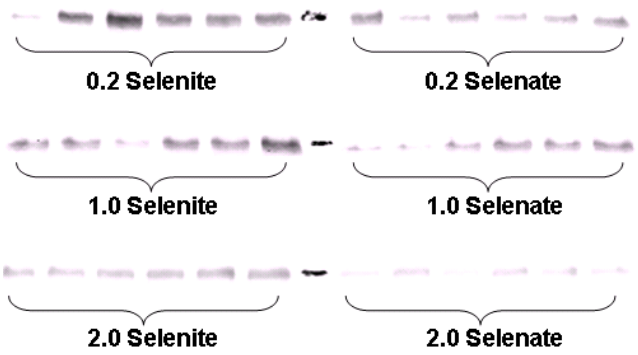


Fig. 2. Regression analyses between liver Se concentration (A, B) or plasma Se concentration (C, D) and native liver PTP activity and PTP glutathionylation due to increasing Se supplementation as selenite (Se IV) [A, C] or selenate (Se VI) [B, D]

A)

Relative OD (Mean ± SD)	Experimental Group		Experimental Group	Relative OD (Mean ± SD)
1,00±0,15	0Se	50 kDa	0.2 Selenite	0,79±0,17
1,00±0,08	0Se	50 kDa	0.2 Selenate	0,41±0,14
1,00±0,09	0Se	50 kDa	1.0 Selenite	0,42±0,15
1,00±0,26	0Se	50 kDa	1.0 Selenate	0,36±0,08
1,00±0,14	0Se	50 kDa	2.0 Selenite	0,39±0,19
1,00±0,30	0Se	50 kDa	2.0 Selenate	0,28±0,09

B)



C)

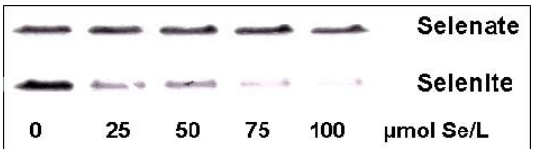


Fig. 3.

A) PTP1B glutathionylation in liver cytosol of rats fed diets containing different amounts of selenite or selenate in comparison to their companions kept on a selenium deficient diet

B) Comparison of PTP1B glutathionylation in liver cytosol of rats fed diets containing selenite or selenate at three dietary levels

C) Glutathionylation of PTP1B after in vitro incubation of liver cytosol with increasing selenite or selenate concentrations – demonstrating that selenate feeding matches in vitro effects of selenite

4. Discussion

By the distinct loss of GPx1 and GPx3 activity due to a lack in Se supply the low rank of these selenoproteins was confirmed by our data [28]. That a Se supply of growing rats with 0.2 mg/kg diet meets their requirements for an abundant selenoprotein synthesis is also in agreement with literature [29]. The fact that an increase in dietary Se concentration effected a dose dependent Se storage in the liver [30] and did not gain in additional selenoprotein synthesis deserves further study into the influence of dispensable Se. Regarding undesirable effects of Se on the development insulin resistant diabetes and obesity our data yield new mechanistic explanations, and the physiological regulation of the insulin antagonistic PTP1B thereby seems to play a central role. In contrast to the mouse trial in which GPx1 overexpression has promoted the development of obesity and insulin resistance [10] our nutrition physiological trial provides explanations by which Se supplements, exceeding the needs, can accelerate these diseases besides a high GPx1 activity. In the mouse trial a decreased tyrosine phosphorylation of the β subunit of the insulin receptor and a decreased phosphorylation of the downstream signalling protein AKT at Thr 308 and Ser 473 has indicated the increased insulin resistance due to GPx1 overexpression [10]. Our current data suggest that the decreased phosphorylation measured in the above mentioned trial reflects more likely an influence of the manipulated Se- and glutathione-dependent redox system on PTP1B than displaying a direct effect of GPx1 on protein phosphorylation. According to our data and to Fig. 4 a lower dietary Se concentration and the resulting higher peroxide concentration due to a lack of GPx1 activity lead to a higher PTP1B inactivation by glutathionylation. Optimised activities of GPx1 by dietary Se (our present study) or an increase in GPx1 expression (mouse study) however remove H_2O_2 and disable PTP1B inhibition [17, 31]. The mentioned aspects therefore provide a plausible explanation for the development of insulin resistance and obesity due to a high GPx1 expression and activity via nutritional Se manipulation. Data of a human study support this hypothesis by the finding that a high erythrocyte GPx1 corresponded to an increased incidence of gestational diabetes [32]. An up-regulation of PTP1B expression and with it an increase in intrinsic insulin resistance could also be found for mice overexpressing catalase, the second central enzyme

in H_2O_2 detoxification [33]. Our data confirm an up-regulation of PTP1B mRNA expression through a high expression of GPx1 as another H_2O_2 detoxifying enzyme [Fig. 1]. In physiological models, like our trial, no overexpression of GPx1 can be obtained by increasing dietary Se concentration beyond the needs. Instead a physiological model of PTP1B regulation manipulating GPx1 expression and activity via a short term Se deficiency could be displayed. Moreover our trial provides information on the influence of Se supply exceeding physiological needs on PTP1B regulation as well as information on the influence of different Se compounds (selenite and selenate) on PTP1B regulation. Since we could show a highly positive correlation between liver- and plasma Se concentration and PTP activity (Fig. 2A-D) our data may be helpful for further analysis of the recent human studies showing a correlation between serum Se and diabetes incidence [11, 12].

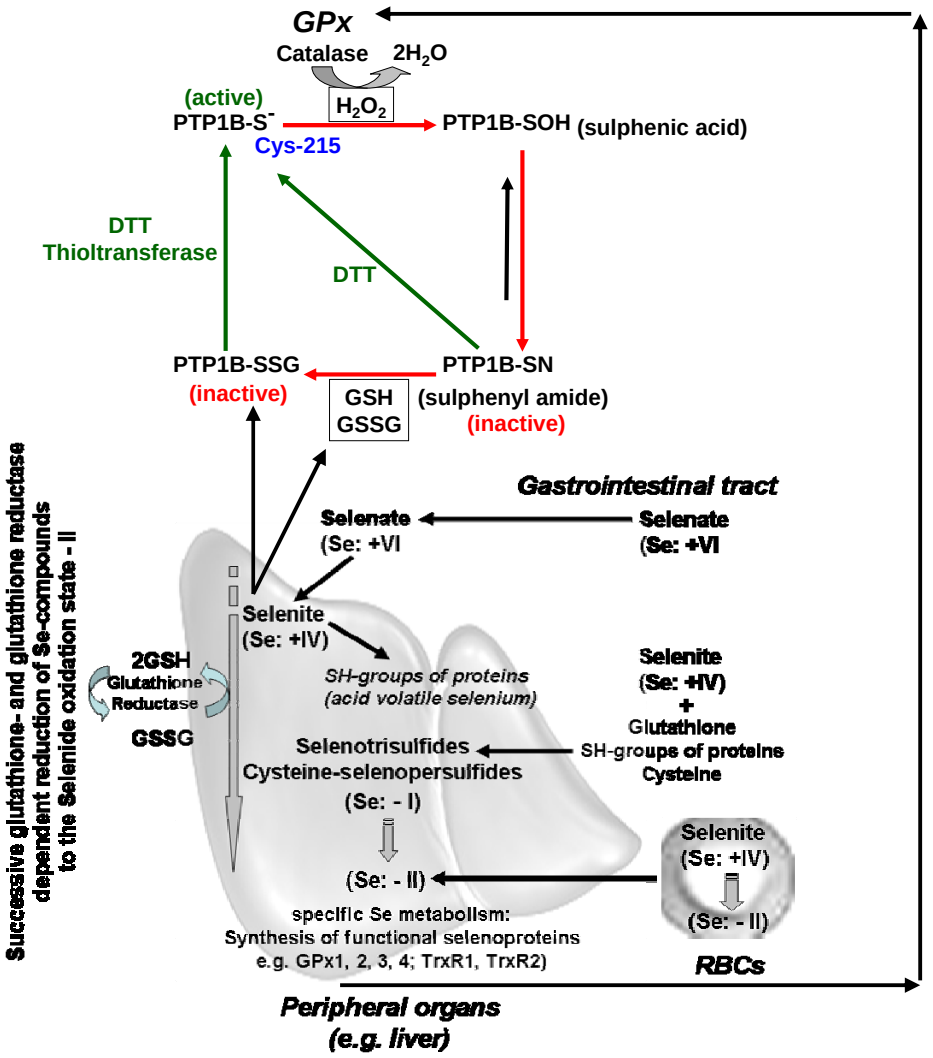


Fig. 4. Current understanding of physiological PTP1B regulation and interfaces with mammalian Se metabolism [according to 8, 17, 30, 31, 34, 35]

In our trial an increase in dietary Se concentration led to a dose dependent increase in PTP activity, corresponding to a loss of PTP1B glutathionylation. Feeding selenate (SeVI) effected a lower PTP1B glutathionylation than feeding selenite (SeIV) (Table 2, Fig. 2, Fig 3B).

This effect presumably derives from fundamental differences in mammalian Se metabolism (Fig. 3). Se from selenite (+IV) and selenate (+VI) is absorbed by individual mechanisms [34] Selenite reacts with thiols like glutathione prior to its absorption and enters the peripheral organs in the form of selenotrisulfides (oxidation state: - I) or it is reduced in the erythrocytes to the selenide oxidation state –II and delivered to peripheral organs bound to albumin [34, 35]. In contrast unmodified selenate can be detected in the bloodstream and in peripheral tissues [35]. During successive selenate reduction the thiol reactive oxidation states (selenite: +IV, and selenotrisulfides: –I) can be formed and require glutathione for their further reduction to the selenide oxidation state (- II) (Fig. 4). Thus glutathione detraction from glutathionylated proteins could be one mechanism for glutathione acquirement. Moreover our data suggest that in vitro effects of selenite (+IV) match selenate (+VI) feeding. This particular aspect of mammalian Se metabolism could be visualized using an in vitro assay (Fig. 3C). Incubation of liver cytosol from group 0Se with increasing selenite or selenate concentrations, representing approximately the Se concentrations in the livers of rats receiving diets with 1.0 and 2.0 mg Se/kg, showed that unreactive selenate (+VI) did not influence PTP1B glutathionylation. In contrast selenite (+IV), matching selenate feeding, effected a dose-dependent loss of PTP1B glutathionylation (Fig. 3C). Thus PTP1B regulation by different Se compounds represents a further important finding of our trial, exceeding the enzymes´ reactions with H_2O_2, reactive oxygen species and glutathione investigated so far [17, 31, 36]. Despite a higher GSSG concentration in the livers of Se supplemented rats in our study a direct PTP1B glutathionylation in the presence of a high GSSG concentration (> 25 mmol/L) [18] could not be confirmed by our data, since millimolar GSSG amounts represent a non-physiological in vitro situation. A manipulation of PTP1B activity causes changes in a number of physiological parameters. In a mouse trial it could be shown, that PTP1B deficient mice had a significantly higher energy expenditure than WT mice [15]. Despite a reduced feed intake in groups 2.0 Selenite and 2.0 Selenate of our trial, possibly

deriving from an impaired palatability of high Se diets [37], the feed conversion ratio (= g feed intake : g body weight gain) was however significantly better in all Se supplemented groups

3.71±0.05 : 1 (0.2 Selenite), 3.73±0.02 : 1 (0.2 Selenate),

3.71±0.03 : 1 (1.0 Selenite), 3.81±0.04 : 1 (1.0 Selenate),

3.80±0.06 : 1 (2.0 Selenite), 3.74±0.03 : 1 (2.0 Selenate)

compared to group 0Se (4.00±0.10 : 1). According to the above mentioned mouse trial [15] the higher feed expense in group 0Se could be an indicator for a higher energy expenditure due to a reduced PTP1B activity. In human studies and in animal trials PTP1B was demonstrated as one factor increasing body weight gain and the development of obesity [13 - 16]. GPx1 overexpressing mice showed a significantly higher body weight and body fat gain [10] whereas mice with a selenoprotein P (SeP) knockout and consequential lacking of peripheral GPx1 synthesis were emaciated [38]. Thus our physiological study was in line with both trials [10, 38], demonstrating that Se supply and high GPx1 activities are involved in body weight and fat gain, and PTP1B regulation may be one factor mediating these effects. Moreover PTP1B and Se were shown as being involved in triglyceride synthesis and storage [39, 40], thus increasing PTP1B activity by a high GPx1 activity and dispensable Se provides a further plausible explanation for the development insulin resistant diabetes and obesity. In conclusion our data could help uncovering mechanisms by which a long-term supranutritional Se supply may have undesirable effects on the development of insulin resistant diabetes and obesity: The regulation of PTP1B expression and activity by Se may be one part of this puzzle. Long term Se supply above the recommendations may be helpful in therapy of prostate cancer [41] and regarding some toxicological aspects where a decreased phosphorylation of critical signalling proteins due to Se supply is desirable [41, 42]. Concerning the development of insulin resistance and obesity a retardation of phosphorylation signals via an increased PTP1B activity is counterproductive and demonstrates the need for future investigations into the differentiated role of antioxidants in metabolic processes.

Acknowledgement

Thank is addressed to: H. Wilhelm Schaumann Foundation, Hamburg (Germany) for financial support. Prof. Dr. Rupert Schmidt (Biotechnical Centre) for advice on RT-PCR experiments and on Western Blot analysis.

Literature

1 Johnson LW, Weinstock RS. The metabolic syndrome: concepts and controversy. Mayo Clin Proc. 2006; 81: 1615–1620
2 Lyons GH , Judson GJ, Ortiz-Monasterio I, Genc Y, Stangoulis JC, Graham RD. Selenium in Australia: selenium status and biofortification of wheat for better health. J Trace Elem Med Biol. 2005; 19: 75-82
3 Broadley MR, White PJ, Bryson RJ, Meacham MC, Bowen HC, Johhnson SE, Hawkesford MJ, McGrath SP, Zhao FJ, Breward N, Harriman M, Tucker M. Biofortification of UK food crops with selenium. Proc Nutr Soc. 2006; 65: 169-181
4 Juniper DT, Philipps RH, Jones AK, Bertin G. Selenium supplementation of lactating dairy cows: effect on selenium concentration in blood milk urine and faeces. J Dairy Sci. 2006; 89: 3544-3551
5 Pappas AC, Acamovic T, Sparks NH, Surai PF, McDevitt RM. Effects of supplementing broiler breeder diets with organoselenium compounds and polyunsaturated fatty acids on egg quality during storage. Poult Sci. 2005; 84: 865 – 874
6 Greenwald P, Anderson D, Nelson SA, Taylor PR. Clinical trials of vitamin and mineral supplements for cancer prevention. Am J Clin Nutr. 2007 Jan;85: 314S-317S
7 Becker DJ, Reul B, Ozcelikay AT, Buchet JP, Henquin JC, Brichard SM. Oral selenate improves glucose homeostasis and partly reverses abnormal expression of liver glycolytic and gluconeogenic enzymes in diabetic rats. Diabetologia 1996: 39: 3-11
8 Mueller AS, Pallauf J. Compendium of the antidiabetic effects of supranutritional selenate doses In vivo and in vitro investigations with type II diabetic dbdb mice. J Nutr Biochem. 2006; 17: 548 – 560
9 Sheng XQ, Huang KX, Xu HB. New experimental observation on the relationship of selenium and diabetes. Biol Trace Elem Res. 2004; 99: 241-253
10 McClung JP, Ronecker CA, Weipeng M, Lisk DJ, Langlais P, Liu F, Lei XG. Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. Proc Natl Acad Sci. 2004; 101: 8852–8857
11 Bleys J, Navas-Acien A, Guallar E. Serum selenium and diabetes in U.S. adults. Diabetes Care. 2007 Apr;30: 829-834
12 Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, Cappuccio FP, Ceriello A, Reid ME. Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. Ann Intern Med. 2007 Aug 21;147: 217-223
13 Dube N, Tremblay ML. Involvement of the small protein tyrosine phosphatases TC-PTP and PTP1B in signal transduction and diseases: From diabetes, obesity to cell cycle and cancer Biochim Biophys Acta 2005; 1754: 108–117
14 Ahmad F Considine RV, Bauer TL, Ohannesian JP, Marco CC, Goldstein BJ. Improved sensitivity to insulin in obese subjects following weight loss is accompanied by reduced protein tyrosine phosphatases in adipose tissue. Metabolism 1997; 46: 1140–1145
15 Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Scarpe AH, Stricker-Krongard A, Shulman GI, Neel BG, Kahn BB. Increased energy expenditure, decreased adiposity and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol. 2000; 20: 5479–5489
16 Mohammad A, Wang J, McNeill JH. Bis(maltolato)oxovanadium (IV) inhibits the activity of PTP1B in Zucker rat skeletal muscle in vivo. Mol Cell Biochem. 2000; 229: 125–128
17 Salmeen A, Andersen JN, Myers MP, Meng TZ, Hinks JA, Tonks NK, Barford D. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. Nature 2003; 423: 769-773
18 Barrett WC, DeGnore JP, Koenig S, Fales HM, Keng YF, Zhang ZY, Yim MB, Chock PB. Regulation of PTP1B via glutathionylation of the active site cysteine 215. Biochemistry 1999; 28: 6699–6705

19 NRC (National Research Council) Nutrient requirements of laboratory animals 4th revised ed, National Academy Press Washington DC 1995
20 Mueller AS, Pallauf J, Most E. Parameters of dietary selenium and vitamin E deficiency in growing rabbits. J Trace Elem Med Biol. 2002; 16: 47–55
21 Tappel ME, Chaudiere J, Tappel AL. Glutathione peroxidase activities of animal tissues. Comp Biochem Physiol. [B] 1982; 73: 945-949
22 Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem. 1982; 106: 207-212
23 Zhu L, Goldstein B. Use of an anaerobic chamber environment for the assay of endogenous cellular protein tyrosine phosphatase activities. Biol Proced Online 2002; 4: 1-9
24 Montalibet J, Skorey KI, Kennedy BP. Protein tyrosine phosphatase: enzymatic assays. Methods 2005; 35: 2-8
25 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970 Aug 15;227(5259):680-685
26 Bradford MM. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72: 248 – 254
27 Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction. Anal Biochem. 1987; 162: 156-159
28 Brigelius-Flohé R Tissue-specific functions of individual glutathione peroxidases Free Radic Biol Med. 2000; 27: 951-965
29 Sunde RA. What can molecular biology tell us about selenium requirements? In: Zimmermann, NG (ed): Proc 3nd Mid-Atlantic Nutrition Conference 2005, University of Maryland, College Park, MD 20742: 8–16
30 Beilstein MA, Whanger PD. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. J Nutr. 1986; 116: 1701-1710
31 Meng TC, Buckley DA, Galic S, Tiganis T, Tonks NK. Regulation of insulin signalling through reversible oxidation of the protein tyrosine phosphatases TC45 and PTP1B. J Biol Chem. 2004; 279: 37716 – 37725
32 Chen X, Scholl TO, Leskiw MJ, Donaldson MR, Stein TP. Association of glutathione peroxidase activity with insulin resistance and dietary fat intake during normal pregnancy. J Clin Endocrinol Metab. 2003; 88: 5963–5968
33 Dong F, Fang CX, Yang X, Zhang X, Lopez FL, Ren J. Cardiac overexpression of catalase rescues cardiac contractile dysfunction induced by insulin resistance: role of oxidative stress, protein carbonyl formation and insulin sensitivity. Diabetologia 2006; 49: 1421–1433
34 Wolffram S, Arduser F, Scharrer E. In vivo intestinal absorption of selenate and selenite by rats. J Nutr. 1985;115: 454–459
35 Suzuki KT, Ohta Y, Suzuki N. Availability and metabolism of 77Se-methylseleninic acid compared simultaneously with those of three related selenocompounds. Toxicol Appl Pharmacol. 2006; 217:51-62
36 von Montfort C, Sharov VS, Metzger S, Schoneich C, Sies H, Klotz LO. Singlet oxygen inactivates protein tyrosine phosphatase 1B by oxidation of the active site cysteine. Biol Chem. 2006; 387: 1399 – 1404
37 Cabe PA, Carmichael NG, Tilson HA. Effects of selenium, alone and in combination with silver or arsenic, in rats. Neurobehav Toxicol. 1979; 1: 275-278
38 Schweizer U, Michaelis M, Koehrle J, Schomburg L. Efficient selenium transfer from mother to offspring in selenoprotein-P-deficient mice enables dose-dependent rescue of phenotypes associated with selenium deficiency. Biochem J. 2004; 378: 21–26
39 Shimizu S, Ugi S, Maegawa H, Egawa K, Nishio Y, Yoshizaki T, Shi K, Nagai Y, Moriono K, Nemoro K, Nakamura T, Bryer-Ash M, Kashiwagi A. Protein Tyrosine Phsophatase 1B as new activator for hepatic lipogenesis via sterol regulatory element-binding protein-1 gene expression. J Biol Chem. 2003; 278: 43095–43101
40 Schaefer K, Kyriakopoulos A, Gessner H, Grune T. Effects of selenium deficiency on fatty acid metabolism in rats fed fish oil-enriched diets. J Trace Elem Med Biol. 2004; 18: 89–97
41 Wu Y, Zu K, Warren MA, Wallace PK, Ip C. Delineating the mechanism by which selenium deactivates Akt in prostate cancer cells. Mol Cancer Therap. 2006; 5: 246 – 252
42 Funchal C, Moretto MB, Vivian L, Zeni G, Rocha JBT, Pessoa-Pureur R. Diphenyl ditelluride- and methylmercury-induced hyperphosphorylation of the high molecular weight neurofilament subunit is prevented by organoselenium compounds in cerebral cortex of young rats. Toxicology 2006; 222: 143 – 153