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

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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. © 2003 Elsevier Inc. All rights reserved.

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 cotranslational 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

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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.

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

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

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 anesthezized 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-

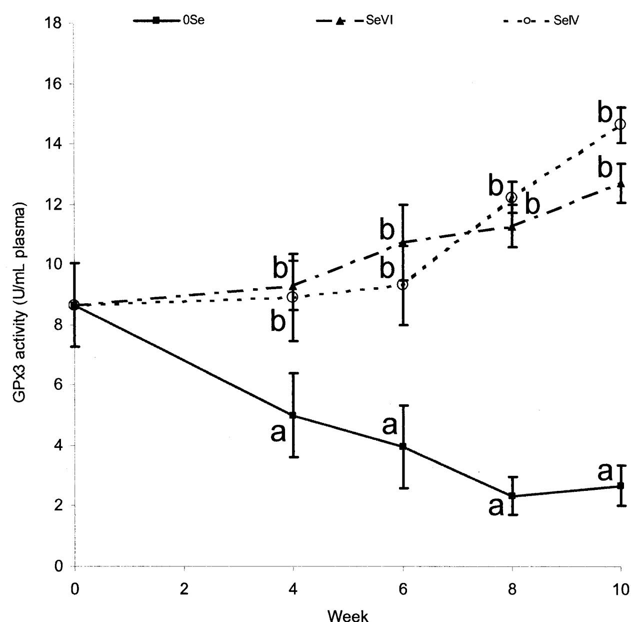


Fig. 1. Significant differences (p<0.05, Tukey test) between groups are indicated by different small letters. Each data point represents the mean±SD of 7 mice per group.

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]

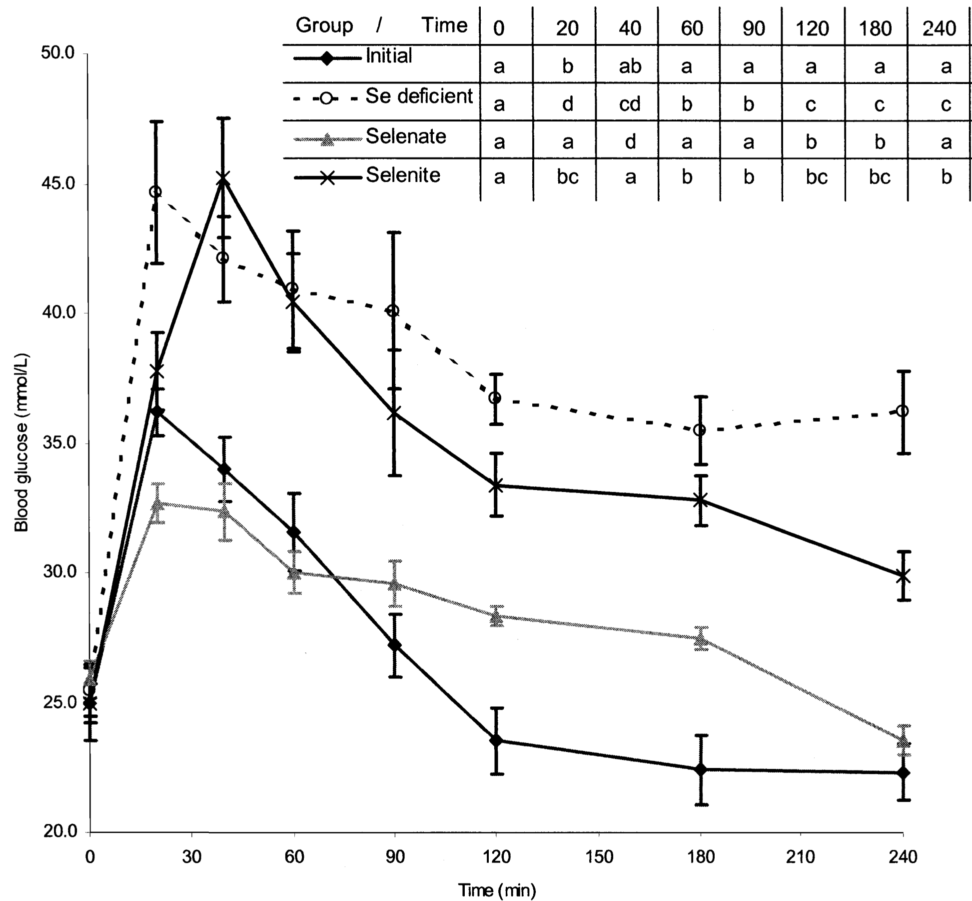
Significant differences (p < 0.05, Turkey test / Dunett-T3 test) between groups are indicated by different superscripts within a line.

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-



Group / Time	0	20	40	60	90	120	180	240
Initial	a	b	ab	a	a	a	a	a
Se deficient	a	d	cd	b	b	c	c	c
Selenate	a	a	d	a	a	b	b	a
Selenite	a	bc	a	b	b	bc	bc	b

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

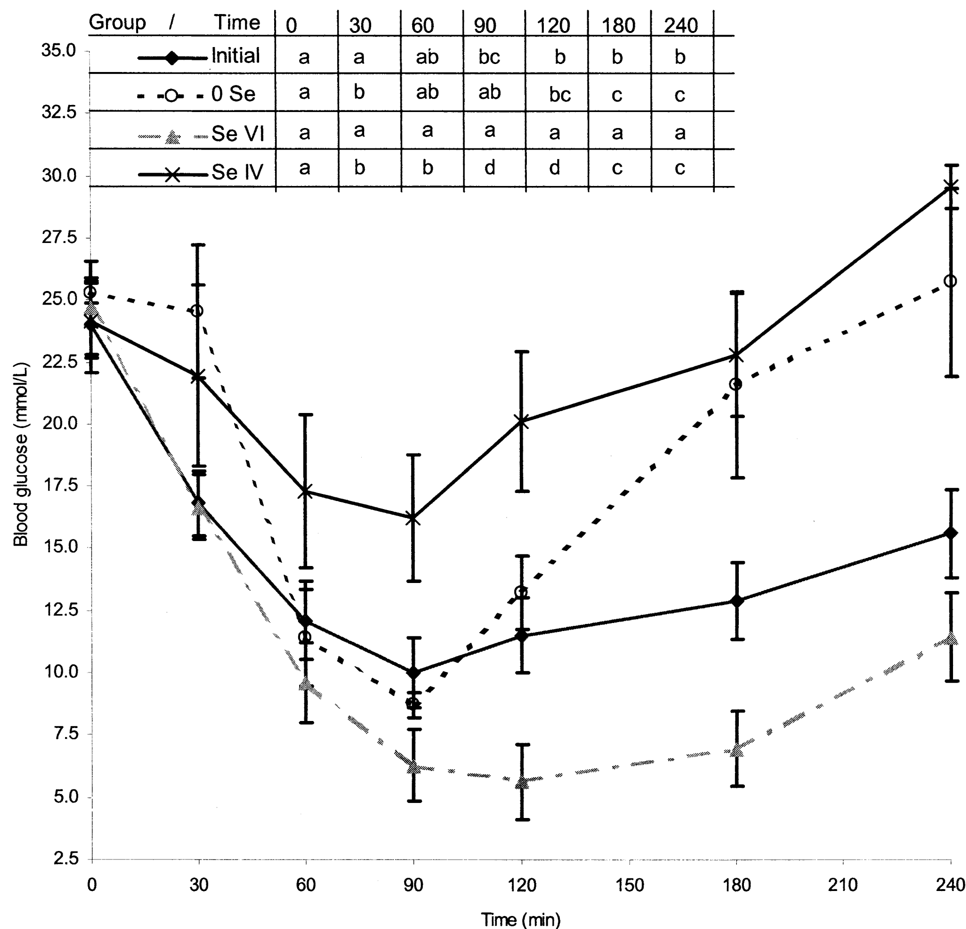


Fig. 2B. Significant differences (p<0.05, Tukey test/Dunnett-T3 test) between groups during IR are indicated by different small letters in the legend table. Each data point represents the mean ± SEM of 7 mice per group.

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.

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