Redox Regulation of Protein Tyrosine Phosphatase 1B by Manipulation of Dietary Selenium Affects the Triglyceride Concentration in Rat Liver

Andreas S. Mueller, Sandra D. Klomann, Nicole M. Wolf, Rupert Schmidt, Julia Spielmann, Gabriele Stangl, Klaus Eder, and Josef Pallauf

Abstract

Protein tyrosine phosphatase 1B (PTP1B) is a key enzyme in the counter-regulation of insulin signaling and in the stimulation of fatty acid synthesis. Selenium (Se), via the activities of glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), is involved in the removal of H2O2 and organic peroxides, which are critical compounds in the modulation of PTP1B activity via glutathionylation. Our study with growing rats investigated how the manipulation of dietary Se concentration influences the regulation of PTP1B and lipogenic effects mediated by PTP1B. Weanling albino rats were divided into 3 groups of 10. The negative control group (NC) was fed a Se-deficient diet for 8 wk. Rats in groups Se75 and Se150 received diets supplemented with 75 or 150 mg Se/kg. Se supplementation of the rats strongly influenced expression and activity of the selenoenzymes cytosolic GPx, plasma GPx, phospholipidhydroperoxide GPx, and cytosolic TrxR, and liver PTP1B. Liver PTP1B activity was significantly higher in groups Se75 and Se150 than in the NC group and this was attributed to a lowered inhibition of the enzyme by glutathionylation. The increased liver PTP1B activity in groups Se75 and Se150 resulted in 1.1- and 1.4-fold higher liver triglyceride concentrations than in the NC rats. The upregulation of the sterol regulatory element binding protein-1c and of fatty acid synthase, 2 PTP1B targets, provided a possible explanation for the lipogenic effect of PTP1B due to the manipulation of dietary Se. We therefore conclude that redox-regulated proteins, such as PTP1B, represent important interfaces between dietary antioxidants such as Se and the regulation of metabolic processes. J. Nutr. 138: 2328–2336, 2008.

Introduction

In recent years, many reports have focused on protein tyrosine phosphatase 1B (PTP1B) as an important target enzyme for the treatment of obesity and diabetes accompanied by insulin resistance (1,2). PTP1B belongs to the cysteine-based phosphatases and antagonizes insulin signaling, because it is capable of dephosphorylating the β-subunit of the insulin receptor and insulin receptor substrate 1 (3,4). In studies with humans (5) and in animal models, a low PTP1B activity, obtained by RNA interference (6–9) or biochemical enzyme inhibition through selenate and vanadium compounds (10–13), protected against obesity, insulin resistance, and diabetes, whereas high PTP1B activities accelerated the development of these diseases. The involvement of PTP1B in fatty acid metabolism is another important physiological function of the enzyme. Feeding fructose-rich diets to rats elevated PTP1B expression and activity, resulting in the induction of fatty acid synthase (FAS) and in an increased liver triglyceride concentration (14,15). The lipogenic mechanism of PTP1B involves the activation of protein phosphatase 2A and subsequently of the sterol regulatory element-binding protein-1c (SREBP-1c) (16,17). This acts as a transcription factor for FAS and other lipogenic enzymes (18,19).

In contrast to PTP1B regulation by exogenously applied inhibitors or RNA interference, the enzyme undergoes a physiological inhibition via oxidation of its active site cysteine residue, Cys-215. In the presence of H2O2, a reversibly oxidized sulphenic acid participates in the inactivation of PTP1B by forming a sulphenic acid in the active site (20).
acid intermediate (PTP1B-SOH) is initially formed (20,21). Its further oxidation can be prevented by the formation of a cyclic sulphenyl amide (22,23), followed by the reaction with reduced and oxidized glutathione (GSH/GSSG) to a mixed disulfide with Cys-215, known as glutathionylation (24). Glutathionylated PTP1B thus represents a catalytically inactive form of the enzyme. Protein glutathionylation of PTP1B and of other enzymes can also take place in the presence of lipid hydroperoxides (25–27). The activity of reversibly oxidized PTP1B and of the glutathionylated enzyme can be partially recovered by the addition of diithiothreitol (DTT) or thiol-transferases (22–24). The direct reaction of the reduced Cys-215 sulphydryl group in the presence of high oxidized glutathione concentrations (>25 mmol/L) may also lead to glutathionylated PTP1B (24). Besides being a by-product of the respiratory chain and being generated by the activity of some oxidoreductases, H2O2 is also produced in mammalian tissues after insulin binding to the insulin receptor, presumably for the differential regulation of PTP activity (28,29). This demonstrates the role of reactive oxygen species, including H2O2, as “second messengers” involved in signal transduction processes (30,31). When taken up at the recommended level (rats, 0.15–0.30 mg Se/kg dietary dry matter; humans, 50–100 μg Se daily), the trace element Se performs its physiological functions in the human and animal body in the form of a catalytically active selenocysteine residue in functional selenoproteins (32). In particular, glutathione peroxidases (GPx) and thioredoxin reductases (TrxR) largely contribute to the maintenance of cellular antioxidant defense. GPx efficiently detoxify H2O2 and organic hydroperoxides (33–36). Besides their important role in the ribonucleotide reductase cycle, TrxR have an unusually wide substrate spectrum. This includes inorganic selenium compounds, dehydroascorbate, and proteins as well as lipid hydroperoxides (37–40). Within the GPx family, cellular GPx (GPx1) exhibits the highest activity and is expressed in all mammalian tissues. Under conditions of dietary Se deficiency, GPx1 as well as plasma GPx (GPx3) undergo a severe loss of enzyme activity accompanied by a distinct down-regulation of their mRNA. Gastrointestinal GPx and phospholipid-hydroperoxide GPx (GPx4) are much more resistant to dietary Se deficiency and therefore rank high in the hierarchy of GPx (35,41,42). Within the TrxR, the cystolic TrxR (TrxR1) is more sensitive to changes in dietary Se supply compared with the mitochondrial enzyme TrxR2 (43). In addition to the microsomal enzyme catalase, which has a major function in H2O2 removal, the selenoperoxidases and TrxR1 are other important enzymes in the reduction of H2O2 and lipid hydroperoxides (33–40,44). In view of the crosslink between physiological PTP1B regulation by H2O2 and lipid hydroperoxides via glutathionylation on the one hand, and the important roles of GPx and TrxR in peroxide metabolism on the other hand, our trial with growing rats examined the following question:

Does a manipulation of dietary Se concentration influence the regulation of PTP1B and PTP1B-dependent lipogenic mechanisms?

## Materials and Methods

### Rats and diets

Thirty healthy growing male albino rats (initial body weight, 78.6 ± 2.69 g) from the Interdisciplinary Research Centre, Department of Animal Nutrition and Nutritional Physiology’s own strain HK51 were randomly assigned to 3 experimental groups of 10. The rats of the Se-deficient negative control group (NC) received a diet based on Torula yeast and Se-deficient wheat (Table 1). The diets for groups Se75 and Se150 were supplemented with sodium selenate to obtain final Se concentrations of 75 μg/kg diet (one-half of the recommended level) and of 150 μg Se/kg diet, representing the recommended level (45–47). The rats were kept individually and had free access to the diet and bidistilled water. After 8 wk, the rats were decapitated under CO2 anesthesia and the livers were excised and prepared for further analysis. The protocol of the animal study was approved by the Regional Council of Giessen.

### Liver total fat, triglycerides, phospholipids, and lipid peroxides

Liver lipids were measured as described previously (11).

Two individual crude fat extracts per rat liver were prepared using a hexane/isopropanol (3:2) mixture containing 0.005% butylated hydroxytoluene. Subsequently, the triglyceride concentration in the lipid extracts was determined in triplicate with a test kit from Bioon (Fluitest TG). The concentration of phospholipids in lipid liver extracts was measured with a test kit after digestion of the samples and liberation of the phospholipid-phosphorus in a mixture of 70% perchloric acid and 30% H2O2. The concentration of liver lipid hydroperoxides was measured using the FOX assay (48). In contrast to the original method, 0.3 g of rat liver were homogenized in ice-cold, HPLC-grade methanol instead of 3.0 g of muscle tissue.

### Se assay

Se concentration in the diets and livers was measured by hydride generation atomic absorption spectrometry (Unicam PU 9400 X; PU 3960 X) as reported previously (49). Certified samples from the National Institute of Standard and Technology (soft winter wheat flour, no. 9438 and bovine liver, no. 1577 b) served as reference material for Se determination in the different matrices.

### Liver GPx1 and GPx4 and plasma GPx3

The activities of GPx1, GPx3, and GPx4 were measured spectrophotometrically (Beckmann DU 50) recording glutathione-dependent peroxide reduction coupled to glutathione reductase and NADPH oxidation (50). For GPx1 activity, the diluted cytosolic supernatants of rat liver homogenates served as sample material, and undiluted rat plasma was used for GPx3. For GPx4 activity, crude homogenates of livers were prepared in a sucrose buffer (51,52). H2O2 served as the substrate for GPx1 and GPx3 determination, whereas freshly synthesized phosphatidylcholine hydroperoxide was used for measurement of GPx4 activity (50–52). One unit of GPx1, GPx3, and GPx4 activity was defined as 1 μmol NADPH oxidized per minute and normalized to 1 mg protein.

### Liver TrxR1

We determined the activity of TrxR spectrophotometrically in the 10,000 × g; 30 min at 4°C cytosolic supernatant of rat liver

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**TABLE 1** Composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torula yeast (Attisholz)</td>
<td>250.00</td>
</tr>
<tr>
<td>Se-deficient wheat (Germany)</td>
<td>100.00</td>
</tr>
<tr>
<td>Cellulose BW 40 (Rettenmaier)</td>
<td>50.00</td>
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<td>Glucose (Sigma-Aldrich)</td>
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<td>Sucrose (Suedzucker)</td>
<td>50.00</td>
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<tr>
<td>Soybean oil (Heiss)</td>
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<tr>
<td>α-Methionine1 (DEGUSSA)</td>
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<tr>
<td>L-Tryptophan (Sigma-Aldrich)</td>
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<tr>
<td>Mineral premix2 (Salts from Sigma-Aldrich)</td>
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</tr>
<tr>
<td>Vitamin premix3 (Vitamins from Roche)</td>
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<tr>
<td>Choline chloride (BASF)</td>
<td>2.00</td>
</tr>
<tr>
<td>Corn starch (Roquette)</td>
<td>398.50</td>
</tr>
<tr>
<td>Selenium premix5</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Total: 1000.00

1 Added according to the recommendations of the NRC (47).
2 Prepared according to the AIN-93G formulation (45,46).
3 Prepared according to the AIN-93G formulation (45,46), with the exception of Se.
4 Prepared according to the AIN-93G formulation (45,46).
5 In diets of groups Se75 and Se150 12.5 g and 25.0 g corn starch were replaced by equal amounts of selenium premix containing 6 mg Se/kg as sodium selenate.
homogenates according to the 5,5′-dithiobis-2-nitrobenzoic acid reduction assay (53). The time-dependent increase in absorption at 412 nm (Beckmann DU 50) based on the formation of 5′-thio-2-nitrobenzoic acid was recorded for 3 min. After subtraction of the absorption obtained in the presence of the TrxR inhibitor aurothioglucose (54), 1 unit of TrxR activity was calculated and defined as 1 μmol 5′-thio-2-nitrobenzoic acid formed per minute and normalized to 1 mg protein.

**Liver PTP.** Differentiated measurement of PTP was carried out using a modified protocol based on para-nitrophenyl phosphate hydrolysis (55,56). For the analysis of PTP activity, 1:5 (wt:v) liver homogenates were prepared under nitrogen gassing in a nonreducing HEPES buffer (50 mMol/L HEPES, 50 mMol/L NaCl, 1 mMol/L EDTA, and 0.1 mMol/L phenylmethylsulphonylfluoride, pH = 7.4). Cytosol was obtained by centrifugation at 10,000 × g; 30 min at 2°C and brought to a final dilution of 1:25 (wt:v). A total of 10 μL of diluted liver cytosol was preincubated at 25°C in 240 μL of HEPES buffer, containing no reducing agents, for 3 min. After the addition of 250 μL HEPES buffer containing 20 mMol/L of the substrate para-nitrophenyl phosphate, samples were incubated for a further 5 min before the reaction was terminated by the addition of 500 μL 2 mol/L NaOH and the absorption was read in a spectrophotometer (Beckmann DU 50) at 410 nm. A blank without cytokos was carried out. Native PTP activity (nonreducing conditions) was calculated using an extinction coefficient of (1.66 × 10° mmol/L)° cm−1 for the paranitrophenolate ion and normalized to 1 mg protein. To determine maximum PTP activity as well as the percentage of glutathionylation (reversible by DTT), enzymatic measurement was repeated as described, but HEPES buffer containing an additional 2.5 mMol/L DTT was used. The percentage of glutathionylated PTP enzyme was calculated from the difference in enzyme activities measured under reducing and nonreducing conditions.

**mRNA expression of liver GPx1, GPx4, TrxR1, SREBP-1c, FAS, and Cyp18.** RNA isolation was carried out using the acid guanidinium thiocyanate phenol chloroform method (57). RT of RNA, followed by PCR for the examination of GPx1, GPx4, TrxR1, SREBP-1c, FAS, and Cyp18 expression in the liver was carried out as described in detail previously (11,32). The gene bank accession numbers and the primers were used as follows: GPx1 (NM0308261); primer forward (5′→3′): TCA TTC AGA ATG TCG GCT CT, primer reverse (5′→3′): CCC ACC AGG AAC TAC TCA AA, amplicon length (bp): 388; GPx4 (NM0171165), primer forward (5′→3′): ATG CAC GAA TTC TCA, primer reverse (5′→3′): GCC AGG TCC TTC TCT AT, amplicon length (bp): 461; TrxR1 (NM031614); primer forward (5′→3′): CCT ATG TGC CCT TGG AAT GT, primer reverse (5′→3′): TGT AGG GCA CAT TGG TCT GC, amplicon length (bp): 390; SREBP-1c (BE63274); primer forward (5′→3′): GCC ACC GAT GAT TGC ACA AT, primer reverse (5′→3′): AGG AAG GCT TCT AGA GAG GA, amplicon length (bp): 158; FAS (NM017332); primer forward (5′→3′): GCC ATC ATT GGG CAC TCT TC, primer reverse (5′→3′): ACC AAC AGC TGC CAT GGA TC, amplicon length (bp): 147; Cyp18 (XM349215); primer forward (5′→3′): ACC ACT GGG GAG AA, primer reverse (5′→3′): AGC CAT TCA GTC TT, amplicon length (bp): 101.

The amplified DNA sequences were visualized with a UV imager (Syngene) after electrophoresis in 1.5% agarose gels containing 0.02 g ethidium bromide/L gel and evaluated in relation to Cyp18 expression using the software Gene Tools from Syngene.

**Western blot analysis of PTP1B glutathionylation.** For analysis of PTP1B glutathionylation, 1:10 (wt:v) liver homogenates were prepared in a nonreducing RIPA lysis buffer (50 mMol/L Tris-HCl, 150 mMol/L NaCl, 1 mMol/L phenylmethylsulphonylfluoride, 1 mMol/L EDTA, 1.0% sodium deoxycholate, 0.1% SDS, and 1% Triton X-100, pH = 7.4). After centrifugation (10,000 × g; 30 min at 2°C), the cytosol was diluted to 1:30 (wt:v). A total of 40 μg of protein was separated according to the standard method (58) but under nonreducing conditions on 15% SDS-polyacrylamide gels (50 mA, 4°C, 2 h). Separated proteins were transferred onto a polyvinylidene difluoride membrane (PALL Biotracte 0.45 μm) by semidy blotting (25 min at constant 6V (60 mA)]. After blocking membranes overnight at 4°C in Tris-buffered saline and Tween (20 mMol/L Tris-HCl, 150 mMol/L NaCl, 0.1% Tween 20, pH = 7.6) containing 5% nonfat dry milk and 0.2% bovine serum albumin, the analysis was continued with a 2-h incubation with the monoclonal anti-glutathione antibody (Virogen 101-A-100) in Tris-buffered saline (1:1500) buffer and a 1-h incubation with the secondary antibody (1:3000) linked to alkaline phosphatase (goat anti-mouse IgG-h+I). Membranes were stained in a reaction buffer (0.1 mol/L Tris, 0.1 mol/L NaCl, 0.05 mol/L MgCl2) containing 0.00375% nitro-blue tetrazolium and 0.0025% 5-bromo-4-chloro-3-indolylphosphatase. Optical density of the −50-kDa PTP1B-band was evaluated (Gene Tools, Syngene) on scanned membranes (CanoScan LiDe 500F).

**Immunohistochemistry and fluorescence microscopy of liver PTP1B.** Small cubic sections (~75 mm3) from the left lobe of freshly dissected livers were embedded in stainless steel wells in a tissue freezing medium (Jung) and frozen in isomyl alcohol, which had been previously cooled in liquid nitrogen (59). The frozen blocks were stored at −80°C until use in fluorescence microscopy. For immunofluorescence, 9-μm sections were prepared using a microtom cryostat (HM 500, Microm) and fixed on slides coated with chrome alaun gelatin (60). The fixed sections were then incubated for 5 min in 70% ethanol, washed for 5 min in PBS, and permeabilized for 10 min in PBS (0.1% Tween 20). After 3 washing steps with PBS, the slides were incubated for 1 h with the monoclonal first antibody (anti-rat-PTP1B, BD Biosciences Pharmingen) and diluted 1:200 in PBS. After being washed 3 times with PBS (PBS, 0.02% Tween), the slides were incubated with the secondary fluorescence antibody (Alexa Flou 488 goat anti-rat IgG, Molecular Probes) for 1 h. The excess second antibody was removed by 3 washes with PBS and 3 washes with PBS. Subsequently, dyeing of the nuclei was carried out by incubating the sections for 30 s in DAPI solution (2 g 4′,6-Diamidino-2-phenylindoldihydrochloride/L PBS). Finally, the sections were covered with Dabco (25 g Dabco/L PBS) and analyzed using an automated fluorescence microscope (Olympus AX 70) equipped with the software “Analysis.” For fluorescence detection of nuclei, an exposure time of 50 ms was chosen. For visualization of PTP1B expression, the exposure time was 1000 ms. All images were taken using a 40 × 2.5 objective, providing an enlargement of 1:100.

**Protein concentration of samples.** The protein concentration of liver cytosol, including samples for Western blotting, was determined using a standard method (61).

**Statistical analysis.** Data are given as means ± SD. Each variable was analyzed in 3 replications. Statistical differences were analyzed with SPSS 14.0 for Windows using the 1-way ANOVA after ascertaining the normality of distribution (Shapiro Wilk test and Kolmogorov Smirnov test) and the homogeneity of variance (Levene test). If the variances were homogenous, the Least Significant Difference test was used to examine significant differences between means. If not, the Games Howell test was used. Differences between means were considered significant at an error probability <5%. For selected variables, an error probability <10% is indicated as a statistical trend. Two-tailed Pearson correlation coefficients and the significance of correlation were analyzed using the correlation mode in SPSS 14.0. Linear regression equations were calculated using Microsoft Excel.

**Results**

**Diets, animal performance, and body weight.** The expected dietary Se concentrations were confirmed by Se analysis (NC, < detection limit of 20; Se75, 78.0 ± 1.57; Se150, 153 ± 1.83 μg Se/kg diet). Calorimetric analysis and protein analysis of the diets revealed that all experimental diets were isoenenergetic and isonitrogenous (NC, 17.5 ± 0.19 MJ/kg, 14.3 ± 0.51% protein; Se75, 17.8 ± 0.11 MJ/kg, 14.1 ± 0.18% protein; Se150, 17.6 ± 0.12 MJ/kg, 14.5 ± 0.29% protein). At the beginning of the experiment body weight did not differ among

2330  Mueller et al.
the NC (78.6 ± 2.46 g), Se75 (78.6 ± 2.91 g), and Se150 (78.6 ± 2.72 g) groups. Total feed intake (TFI) over 8 wk and feed efficiency ratio (FER; g feed intake:g body weight gain) in group NC (TFI, 945 ± 59.0 g; FER, 4.56 ± 0.26) were significantly impaired compared with the Se75 (TFI, 1066 ± 63.8 g; FER, 4.22 ± 0.23) and Se150 (TFI, 1014 ± 48.6 g; FER, 4.33 ± 0.19) groups. Final body weight in Se75 (331 ± 23.4 g) and Se150 (314 ± 21.3 g) groups was significantly higher than in the NC group (287 ± 24.9 g).

Se status. The liver Se concentration in the NC was reduced to 2.2 and 1.7% of the concentrations in the Se75 and Se150 groups (Table 2). Rats in group Se150 had a liver Se concentration that was greater than that in Se75 rats. Eight weeks of Se deficiency led to the most distinct loss of activity of liver GPx1 and of GPx3. In the NC group, liver GPx1 activity was 99% lower than in the Se75 and Se150 groups and was accompanied by a ~5.5-fold downregulation of GPx1 mRNA. Raising the dietary Se concentration from 75 to 150 mg/kg tended to increase liver GPx1 activity (P = 0.08). The 98% reduction of GPx3 in the NC group compared with rats in the Se75 and Se150 groups was similar to that for liver GPx1. However, the mRNA abundance and the activity of liver GPx4 and liver TrxR1 were distinctly less affected by the lack of dietary Se. Compared with groups Se75 and Se150, GPx4 mRNA in the NC group was downregulated by 23 and 45% and resulted in a reduced GPx4 activity to 34% and 22%, respectively. The 1.8-fold and 2.0-fold downregulation of liver TrxR1 mRNA abundance resulted in reductions in enzyme activity to 40 and 30%, respectively, in the NC group compared with the Se75 and Se150 groups.

Regulation of liver PTP1B. The effective liver PTP activity, measured under native, nonreducing conditions (without DTT), was 1.3- higher in the Se75 group and 1.9-fold higher in the Se150 group compared with the NC group (Fig. 1A). Measurement of PTP activity under reducing conditions (with DTT added) indicated the regeneration of enzyme activity inhibited by glutathionylation. Under reducing conditions, the increase in liver PTP activity in group NC (+105%) was greater than in the Se75 (+24%) and Se150 (+19%) groups. PTP inhibition by glutathionylation, calculated from the ratio of PTP activity under native and reducing conditions, was significantly higher in NC rats (50.1 ± 7.76%) than in Se75 (23.4 ± 8.17%) and Se150 (15.5 ± 10.7%) rats. Under reducing conditions, PTP activity was still significantly lower in NC rats (1.69 ± 0.37 U/mg protein) than in the Se75 (2.37 ± 0.49 U/mg protein) and Se150 (2.99 ± 0.42 U/mg protein) groups. However, the factors for activity difference between the NC group and the Se75 (0.4-fold) and Se150 (0.8-fold) groups were significantly lower than the native conditions (1.4- and 2.0-fold). The remaining differences in PTP activity between the experimental groups can therefore be explained by changes in PTP expression due to dietary Se. They could be visualized using immunofluorescence (Fig. 1B). PTP1B expression was distinctly higher in group Se150 than in the NC group. Post-transcriptional PTP1B regulation via glutathionylation could be depicted by Western blot analysis using an anti-glutathione antibody (Fig. 1C). In Western blot analysis (Fig. 1C), a significantly higher PTP1B glutathionylation was measured in NC rats than in Se 75 (1.9-fold) and Se 150 (2.3-fold) rats, demonstrating increased PTP1B inhibition by glutathionylation due to Se deficiency.

Liver total fat, triglyceride, phospholipid, and lipid hydroperoxide concentrations. Total liver fat concentration was significantly higher in groups Se75 (14%) and Se150 (21%) than in the NC group (Table 3). Se150 rats tended to have a higher liver total fat concentration than Se75 rats (P = 0.06). Liver triglyceride concentrations were significantly higher in the Se75 (1.1-fold) and Se150 (1.4-fold) groups than in the NC group. Se150 rats had a significantly higher liver triglyceride concentration than Se75 rats. This effect also remained uninfluenced when liver triglyceride concentration was related to 1 g of final body weight. In contrast, the liver phospholipid concentration was uninfluenced by dietary Se (NC, 192 ± 19.1; Se75, 183 ± 16.9; Se150, 193 ± 14.2 μmol/g dry matter). The livers of NC rats had a significantly higher lipid hydroperoxide concentration than those of the Se75 (0.7-fold) and Se150 (2.0-fold) rats.

mRNA expression of SREBP-1c and FAS. To further explain the chain of cause and effect linking Se-dependent PTP1B redox regulation to triglyceride metabolism, we subsequently investigated the expression of 2 key genes involved in FAS. We analyzed a significantly higher expression of liver SREBP-1c in the Se75 (1.2-fold) and Se150 (1.3-fold) groups than in the NC group (Fig. 2). FAS, a target of the transcription factor SREBP-1c, was expressed significantly higher in livers of Se75 (0.6-fold) and Se150 (0.9-fold) rats than in NC rats (Fig. 2).

Correlation and regression analyses between dietary Se, antioxidant selenoenzymes, PTP1B regulation, and liver triglyceride concentration. PTP1B is an accepted molecular trigger of triglyceride synthesis via SREBP-1c and FAS. Because our present data showed a distinct regulation of PTP1B due to a manipulation of dietary Se and Se-dependent antioxidant enzymes, we consequently examined possible molecular links between dietary Se supply, the resulting activity of antioxidant selenoenzymes, the redox regulation of PTP1B, and liver triglyceride concentration by correlation and regression analyses.

Liver Se concentration (Fig. 3A) as well as the activity of liver GPx1, GPx4, TrxR1, and of GPx3 (Table 4) were positively correlated (P < 0.01) with native liver PTP activity and liver triglyceride concentration. Whereas the native PTP activity correlated positively with liver triglyceride concentration (P <

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**TABLE 2** Liver Se concentration, liver GPx1, GPx4, and TrxR1 activity and expression, and GPx3 activity of rats fed diets containing 0, 75, and 150 μg Se/kg for 8 wk

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>Se75</th>
<th>Se150</th>
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</thead>
<tbody>
<tr>
<td>Liver Se concentration, nmol/g dry matter</td>
<td>0.66 ± 0.11a</td>
<td>28.7 ± 1.83b</td>
<td>36.9 ± 2.71b</td>
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<td>Liver GPx1 activity, μU/mg protein</td>
<td>8.73 ± 2.25b</td>
<td>722 ± 147a</td>
<td>831 ± 139a</td>
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<td>mRNA, fold of NC</td>
<td>1.00 ± 0.17b</td>
<td>6.11 ± 0.44a</td>
<td>6.81 ± 0.82a</td>
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<td>Liver GPx4 activity, μU/mg protein</td>
<td>1.41 ± 0.71c</td>
<td>4.16 ± 0.81b</td>
<td>6.31 ± 1.85b</td>
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<td>mRNA, fold of NC</td>
<td>1.00 ± 0.09d</td>
<td>1.23 ± 0.11b</td>
<td>1.45 ± 0.20a</td>
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<tr>
<td>Liver TrxR1 activity, μU/mg protein</td>
<td>4.02 ± 0.45c</td>
<td>10.0 ± 0.87b</td>
<td>13.5 ± 2.81b</td>
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<td>mRNA, fold of NC</td>
<td>1.00 ± 0.10b</td>
<td>2.82 ± 0.14a</td>
<td>3.04 ± 0.26a</td>
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<td>GPx3 activity, μU/mg protein</td>
<td>0.62 ± 0.17b</td>
<td>25.0 ± 5.48a</td>
<td>31.0 ± 7.48a</td>
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</table>

1 Values are means ± SD, n = 10. Means in a row without a common letter differ, P < 0.05.
0.01), PTP glutathionylation, indicating PTP inhibition, and liver triglyceride concentration were negatively correlated \((P < 0.01)\) (Fig. 3B). All interrelationships examined fit well to linear regression lines.

**Discussion**

Our current results on the regulation of the functional selenoproteins in rats are in accordance with data from the literature. The dramatic loss of GPx1 mRNA abundance and enzyme activity due to a lack of dietary Se supply indicates the low rank of GPx1 in the hierarchy of functional selenoproteins (62,63). GPx3 was also strongly affected by Se deficiency and therefore is the second-lowest ranking selenoperoxidase (35). GPx4 with a much lower activity in rat liver than GPx1 and TrxR1 are much less affected by Se deficiency with regard to mRNA abundance and enzyme activity (41,43,64,65). GPx4 and TrxR1 therefore have a high ranking in the selenoprotein hierarchy. Our data confirm that a Se supply of 150 \(\mu g/\)kg diet meets the requirements of growing rats for an abundant synthesis of GPx1, GPx3, GPx4, and of TrxR1 (43,62,63,65). Only a slight further increase in mRNA abundance and enzyme activity was achieved for all

**TABLE 3** Liver total fat, triglycerides, and lipid hydroperoxides of rats fed diets containing 0, 75, and 150 \(\mu g\) Se/kg for 8 wk

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>Se75</th>
<th>Se150</th>
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</thead>
<tbody>
<tr>
<td>Total fat, % in dry matter</td>
<td>16.8 ± 2.19(^a)</td>
<td>19.5 ± 2.39(^b)</td>
<td>21.2 ± 2.43(^b)</td>
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<td>Triglycerides, (\mu mol/g) dry matter</td>
<td>52.7 ± 14.1(^c)</td>
<td>111 ± 22.8(^b)</td>
<td>127 ± 29.5(^c)</td>
</tr>
<tr>
<td>Triglycerides, (\mu mol/g) body weight</td>
<td>0.16 ± 0.015(^c)</td>
<td>0.33 ± 0.025(^b)</td>
<td>0.41 ± 0.028(^b)</td>
</tr>
<tr>
<td>Lipid hydroperoxides, nmol/ (\mu mol) liver triglycerides</td>
<td>2.53 ± 0.64(^b)</td>
<td>1.48 ± 0.20(^b)</td>
<td>0.83 ± 0.29(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Values are means ± SD, \(n = 10\). Means in a row without a common letter differ, \(P < 0.05\).
Liver triglyceride concentration and native liver PTP activity and liver triglyceride concentration

Table 4

<table>
<thead>
<tr>
<th>Correlation investigated</th>
<th>Pearson coefficient</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx1 native PTP</td>
<td>0.95</td>
<td>y = 0.0019 x + 0.74</td>
</tr>
<tr>
<td>GPx3 native PTP</td>
<td>0.96</td>
<td>y = 0.051 x + 0.75</td>
</tr>
<tr>
<td>GPx5 native PTP</td>
<td>0.92</td>
<td>y = 0.203 x + 0.79</td>
</tr>
<tr>
<td>TNFR1 native PTP</td>
<td>0.93</td>
<td>y = 0.166 x + 0.18</td>
</tr>
<tr>
<td>GPx1 liver TG</td>
<td>0.96</td>
<td>y = 0.031 x + 15.0</td>
</tr>
<tr>
<td>GPx3 liver TG</td>
<td>0.97</td>
<td>y = 0.832 x + 15.4</td>
</tr>
<tr>
<td>GPx5 liver TG</td>
<td>0.90</td>
<td>y = 3.195 x + 7.04</td>
</tr>
<tr>
<td>TNFR1 liver TG</td>
<td>0.94</td>
<td>y = 2.747 x + 5.76</td>
</tr>
</tbody>
</table>

Analyses were based on n = 30 rats fed diets containing 0, 75, and 150 μg Se/kg for 8 wk. All correlations were significant, P < 0.01.

FIGURE 3  (A) Linear correlation and regression analyses investigating the interrelationship between liver Se concentration and liver triglyceride concentration and native PTP activity. (B) The interrelationship between liver triglyceride concentration and PTP activity and glutathionylation. Analyses were based on 30 rats fed diets containing 0, 75, and 150 μg Se/kg for 8 wk. All correlations were significant, P < 0.01.

selenoproteins investigated by raising the dietary Se concentration from 75 μg/kg diet to 150 μg/kg diet. Our results, moreover, demonstrate an additional role of Se and antioxidant selenoproteins in the regulation of metabolic processes, which exceeds a purely antioxidant function. The inhibition of PTP1B activity by H2O2 and lipid hydroperoxides followed by glutathionylation and a subsequent partial regeneration of PTP1B activity with DTT has, as yet, only been shown in vitro (20–24,27). These mechanisms are physiologically relevant and influenced by Se and the antioxidant selenoenzymes GPx1, GPx3, GPx4, and TrxR1. On the one hand, higher peroxide concentrations (Table 3) resulting from a low dietary Se concentration and lowered activities of the antioxidant selenoproteins lead to a reduced activity of PTP1B via glutathionylation (Table 2; Fig. 1A,C). On the other hand, increased expression and optimized activities of GPx1, GPx3, GPx4, and TrxR1, which participate in the metabolism of H2O2 and organic peroxides, can be assumed to lower peroxide tone and disable PTP1B inhibition (33,34,39,66,67). A similar connection between a H2O2-detoxifying enzyme and PTP1B regulation, as suggested by our data, has also already been demonstrated for catalase. However, most of the experiments, in which the inactivation of PTP1B by reactive oxygen species could be prevented by catalase, were also carried out only in vitro (68–70). In addition to post-transcriptional regulation of PTP1B by glutathionylation, our results also indicate a changed expression of PTP1B due to dietary Se supply. This transcriptional regulation is reflected in the difference in PTP activity that still exists among the experimental groups after DTT treatment and in the protein expression examined by immunofluorescence (Fig. 1A,B). This finding of an increased PTP1B expression is in accordance with data from a mouse study in which the overexpression of catalase, another H2O2-detoxifying enzyme, led to a 3-fold elevated PTP1B expression (71). An altered PTP1B activity has a number of additional metabolic consequences. Lipid metabolism seems to represent one particular target of PTP1B. An upregulated PTP1B activity activates lipogenic mechanisms, involving the activation of SREBP-1c and of its target gene, FAS, as was shown originally for rats fed fructose-enriched diets (14–17). Moreover, PTP1B expression is a valuable indicator for the diagnosis of nonalcoholic-fatty-liver-disease, a disorder closely related to hepatic insulin resistance and metabolic syndrome (72–74). However, the manipulation of PTP1B by dietary Se powerfully influences liver lipid metabolism. Simultaneously, we could confirm that the higher liver triglyceride concentrations due to the Se-dependent upregulation of PTP1B are based on the activation of SREBP-1c and FAS (18,19), as was described for fructose feeding (14–17). The existence of a relationship between liver lipid metabolism and antioxidant selenoproteins was also demonstrated by an inverse experimental setup (75). In this study, the overexpression of SREBP-1c in fibroblasts and hepatocytes led to a manifold upregulation of GPx3. This result corresponds to the highest correlation between GPx3 and liver triglyceride concentration in our study (Table 4). Liver triglyceride metabolism represents a Se- and PTP1B-dependent target and does not mainly depend on changes in feed intake and body weight gain. Despite a higher feed intake and a higher body weight in the Se75 group compared with the Se150 group, liver triglyceride concentration was higher in the Se150 group and was accompanied by the most effective PTP activity. Figure 4 summarizes our hypotheses regarding the influence of Se on liver triglyceride metabolism. Nevertheless, PTP1B regulation by dietary Se seems to influence metabolic processes variously. In a mouse trial, PTP1B-deficient mice had a significantly higher energy expenditure than wild-type mice (7).
Se supplements ↑
Antioxidant selenoenzymes (GPx1, GPx3, GPx4, TrxR1) ↑

PTP1B

SSG

PTP1B

(active)

S–

PP2A

SREBP1-c

Fatty acid synthase

Triglyceride synthesis

FIGURE 4 The possible molecular link between dietary Se and liver triglyceride metabolism involves the differential regulation of PTP1B. Checkmarks indicate checkpoints investigated in the present study.

In our trial, the feed conversion ratio (g feed:g body weight gain) was better in the Se-supplemented groups than in the Se-deficient rats of the NC group. The higher feed expense in the Se-deficient NC group may therefore be an indicator for a higher energy expenditure due to the reduced PTP1B activity. Data of a very recent study even suggest an important role for hypothalamic PTP1B in the reduction of feed intake and body fat stores (76). In human studies and in animal trials, PTP1B was demonstrated as one factor increasing body weight gain and the development of obesity (1,2,6–9). GPx1-overexpressing mice had a significantly higher body weight and body fat gain (77,78), whereas mice with a selenoprotein P knockout and the resulting lack of antioxidant selenoprotein synthesis were emaciated (79). Thus, our results regarding body weight are in line with both trials (77–79), demonstrating that Se supply and high activities of antioxidant selenoproteins seem to have a function in body weight development and in lipid metabolism.

Our findings, demonstrating a distinct influence of dietary Se supplementation on the redox regulation of the insulin-antagonistic PTP1B and on metabolic processes such as lipid metabolism, are of importance in assessing the benefits and risks of Se supplements. The mouse study, in which GPx1 overexpression led to the development of obesity and diabetes (77,78) as well as most recent data from epidemiological human studies in which a high Se status increased the diabetes risk and effected an elevation of serum lipids (80–83), highlight the ambivalent role of antioxidants such as Se in metabolic processes and in the development of metabolic disorders.

The influence of physical activity is also important in the context of antioxidant supplementation. In our rat trial, the lower body weight and liver triglyceride concentration in NC rats may have derived from the reduced PTP1B activity due to artificial oxidative stress caused by Se deficiency. In a trial with diet-induced obese rats, oxidative stress resulting from a single bout of exercise effectively reduced PTP1B and improved insulin sensitivity (84). Thus, reduced physical activity combined with a high antioxidant supplementation may explain even undesirable effects of antioxidants in the development of diseases of civilization.

In conclusion, it appears that redox-regulated proteins, such as PTP1B, which are involved in cellular signaling processes represent important interfaces between dietary antioxidants such as Se and metabolic processes (85). The involvement of PTP1B in the development of insulin resistance, obesity, and fatty liver disease on the one hand (1,2,6–9,72–74) and very recent critical observations regarding the relation between Se, obesity, elevated serum lipids, and diabetes (77,80–82) on the other strongly suggest the necessity for future research of regulatory functions of Se and other antioxidants in metabolic processes and metabolic disorders.

Literature Cited


73. Sanal MG. The blind men ‘see’ the elephant: the many faces of fatty liver disease. World J Gastroenterol. 2008;14:831–44


