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Dietary selenium regulation of glutathione peroxidase mRNA and other selenium-dependent parameters in male rats

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Weanling male rats were fed a basal torula yeast diet (0.007 μg Se/g diet) supplemented with graded levels of Se (0 to 0.2 μg Se/g diet as Na_2SeO_3) (three rats/group) to evaluate classical glutathione peroxidase (GPX1, GSH:H_2O_2 oxidoreductase, EC 1.11.1.9) mRNA level as an indicator of intracellular Se status. Growth was followed throughout the dietary treatment and a number of Se-dependent parameters including liver GPX1 mRNA levels were determined after 33 days. Growth was not impaired at any level of dietary Se supplementation. In rats fed the Se-deficient basal diet, liver Se concentration was 5 ± 1%, liver GPX1 mRNA levels were 10 ± 2%, plasma GPX activity was 2 ± 1%, erythrocyte GPX activity was 37 ± 1%, and liver GPX activity was 0 ± 2% of the levels in rats fed 0.1 μg Se/g diet; these parameters increased sigmoidally with increasing dietary Se, showing a breakpoint near 0.1 μg Se/g diet. Graphical analysis indicated that the increase in liver GPX1 mRNA level with increasing dietary Se, preceded the increase in liver GPX activity. Se supplementation had no effect on polyadenylated mRNA levels or on β-actin mRNA levels, demonstrating that Se regulation of GPX1 mRNA is specific. Se-deficient liver selenoprotein P mRNA levels were 69 ± 2% of the levels in rats fed 0.1 μg Se/g diet. We hypothesize that GPX1 mRNA is a primary target of the Se regulatory mechanism, making GPX1 mRNA level a potentially useful indicator of the status of an important intracellular regulatory pool of Se. (J. Nutr. Biochem. 8:85–91, 1997.) © *Elsevier Science Inc. 1997*

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Introduction

In tissues such as rat liver, classical glutathione peroxidase (GPX or GPX1, glutathione: H_2O_2 oxidoreductase E.C. 1.11.1.9) activity virtually disappears in Se deficiency,[1] and liver GPX activity increases with increasing dietary Se between 0.02 and 0.1 μg Se/g diet.[2] This dramatic response of GPX activity to Se status has provided a most useful parameter for determining dietary Se requirements (for review, see ref. 3). In general, minimal dietary Se requirements for most species are at or near 0.1 μg Se/g diet when based on GPX activity, suggesting that Se regulation of this enzyme has been highly conserved.[3,4] When parameters other than GPX activity are used, estimated Se requirements can be higher or lower than 0.1 μg Se/g diet. For example, the Se requirement for prevention of Keshan disease in humans is approximately half of the Se requirement for maximal GPX activity.[5] Likewise, selenoproteins such as phospholipid hydroperoxide glutathione peroxidase have been shown to require less than 0.1 μg Se/g diet to reach maximal levels.[6,7] In contrast turkeys require more than 0.1 μg Se/g diet to prevent gizzard myopathy[8] and male rats fed 0.2 μg Se/g diet were shown to have greater protection against retinal capillary degeneration when compared to male rats fed the 0.1 μg Se/g diet.[9] Thus most recent recommendations for dietary Se requirements often include a margin of

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safety above the minimum requirement for GPX activity.[10] In light of this abundance of new information, it is important to identify the most appropriate parameters for determining dietary Se requirements.

In addition to GPX activity, GPX protein levels also fall to 20% or less in Se deficiency,[11–13] suggesting that the response of GPX to dietary Se status could be the result of a mechanism that regulates GPX synthesis. Consistent with this idea, it has become clear that Se status also has a dramatic effect on the level of GPX1 mRNA. In rats fed a Se-deficient diet, liver GPX1 mRNA levels decrease exponentially to approximately 10% of Se-adequate levels.[2,6,14–17] Several studies have shown that GPX1 transcription is not affected by Se status,[17–19] indicating that Se status must regulate GPX1 mRNA stability. We have recently demonstrated that the GPX1 3′ untranslated region (3′UTR) is necessary for GPX1 mRNA levels to decrease in Se-deficient cells,[20] suggesting that the GPX1 3′UTR may contain an Se-responsive element.

We hypothesize that GPX1 mRNA levels are linked to a cellular Se thermostat, making GPX1 mRNA levels a sensitive indicator of intracellular Se status. The purpose of the present experiment was to critically evaluate GPX1 mRNA level as a parameter that can be used to evaluate and understand Se requirements, as well as to gain insight into the to mechanism for Se regulation of GPX1 expression.

Methods and materials

Animals and diets

Male weanling rats, 21 days old, were obtained from Holtzman (Madison, WI USA) and housed individually in hanging wire-mesh cages. The initial body weights ranged from 60 to 65 g. The care and treatment protocol of the experimental animals was approved by the Institutional Animal Care and Use Committee at the University of Missouri. Rats were fed the basal diet,* which was the torula-yeast diet described by Knight and Sunde[11] supplemented with 0.4 g/100 g D,L-methionine (U.S. Biochemical, Cleveland, OH USA) and 100 mg/kg all-*rac*-α-tocopheryl acetate to allow adequate growth and to prevent liver necrosis, respectively. Neutron activation analysis showed that the basal diet contained 0.007 μg Se/g diet. Rats were fed the basal diet alone or that diet supplemented with Se as Na_2SeO_3. Rats were given free access to food and deionized water at all times. All named chemicals, unless otherwise indicated, were purchased from Sigma Chemical (St. Louis, MO USA).

Experimental design

The weanling rats were randomly assigned to one of six dietary treatment groups, three rats per group. Each group was fed the basal diet supplemented with 0, 0.02, 0.05, 0.1, 0.15, or 0.2 μg Se/g diet as Na_2SeO_3. During the 33-day dietary treatment, rat body weights were recorded twice per week. At the end of the experiment, rats were anesthetized with ether, blood samples were taken by cardiac puncture using a heparinized syringe, and livers were perfused in situ with ice-cold 0.15 mol/L KCl to flush out erythrocytes. Blood and liver were stored on ice, centrifuged and homogenized at 4°C, and analyzed immediately for GPX activity and protein. A portion of each liver was frozen immediately in liquid nitrogen, then stored at −80°C so that RNA could be isolated from the tissue. For GPX activity analysis, plasma and erythrocytes were separated by centrifugation (1400 × g, 15 min, GPR tabletop centrifuge, GH-3.7 rotor, Beckman Instruments, Palo Alto, CA USA). Approximately 1 g of each large left liver lobe was homogenized in 9 vol of 0.25 mol/L sucrose containing 0.25 mmol/L EDTA. The homogenate was centrifuged (7800 × g, 20 min, Model J-21C, JA-20 rotor, Beckman Instruments, Palo Alto, CA USA), and the supernatant was recentrifuged (105,000 × g, 60 min, Model L8-M, rotor 70.1 Ti, Beckman Instruments, Palo Alto, CA USA). GPX activity was determined for plasma, erythrocytes, and liver cytosol by the coupled assay procedure[21] using H_2O_2, so that only Se-dependent GPX activity was measured. Protein was measured by the Lowry assay[22] so that enzyme units could be expressed per gram of protein. Neutron activation analysis[23] was used to determine Se concentrations in liver tissue samples and in the experimental diets.

Northern blot analysis

Total RNA was isolated from 1-g portions of liver by homogenization in guanidine isothiocyanate lysis buffer followed by centrifugation (20 hr) on 5.7 mol/L CsCl as described previously.[14] The RNA pellet was dissolved in diethyl pyrocarbonate-treated water and quantitated spectrophotometrically by A_{260} (ϵ = 25 mL · mg^{-1} · cm^{-1}). Total RNA (30 μg each sample) was fractionated by formaldehyde-denaturing agarose gel electrophoresis, stained with acridine orange, and capillary transferred to modified nylon 66 membranes (Biotrans nylon membrane, ICN, Irvine, CA USA). Northern blots were hybridized with the ^{32}P-labeled 0.7 kb probe for murine GPX1[14] (Oligolabeling kit, Pharmacia LKB, Piscataway, NJ USA and [α-^{32}P] dCTP DuPont NEN, Wilmington, DE USA). Autoradiograms were scanned with an LKB 2222 UltraScan XL Laser Densitometer (Pharmacia LKB Biotechnology, Uppsala, Sweden) and the peak areas were quantified using the GelScan XL program. The GPX1 mRNA level for each dietary treatment was determined as a relative percentage of the 0.1 μg Se/g mRNA peak area within that set of samples. The three replicate peak area percentages for each dietary treatment were averaged to determine the mean GPX1 mRNA levels.

Relative mRNA analysis

The relative level of polyadenylated (poly A) RNA in each sample of total RNA was determined by slot-blot hybridization using synthetic ^{35}S-labeled polythymidylate (poly dT) as a probe as described.[24] Membranes were washed twice in PESB (40 mmol/L sodium phosphate, pH 7, 1 mmol/L EDTA, 50 g SDS/L, 5 g BSA/L) at 25°C for 10 min, and three times in PES (40 mmol/L sodium phosphate, pH 7, 10 g SDS/L, 1 mmol/L EDTA) at 44°C for 10 min. The blots were dried, and individual slots were counted in scintillation vials (1900 TR, Packard Instrument Co., Downers Grove, IL USA) containing 3 mL of scintillant (Packard Ultima Gold).

Slot blot analysis

Total RNA slot loads were adjusted so that each sample contained an equal amount of poly A RNA (~7 μg total RNA). RNA was denatured and vacuum-transferred (PR600, Hoeffer Scientific Instruments, San Francisco, CA USA) to modified nylon 66 membranes (Biotrans nylon membrane, ICN, Irvine, CA USA) as described previously.[24] The slot-blot membranes were baked 1 to 2 hr at 80°C and then hybridized sequentially with the 0.7-kb murine GPX1 probe, a 1.2-kb BglI probe fragment containing the central portion of the rat β-actin cDNA, and a 2.2-kb EcoRI/HindIII fragment containing the 5′ portion of the rat selenoprotein P cDNA

*Basal torula yeast diet (g/kg diet, as described by Knight & Sunde 1987): torula yeast, 300; sucrose, 585.9; lard, 50; mineral mix, 50; vitamin mix, 9; D,L-methionine, 4; all-*rac*-α-tocopheryl acetate, 0.1; choline chloride, 1.

kindly provided by Dr. Hill and Dr. Burk,[25] as described for Northern analysis. After each hybridization, autoradiograms were quantitated by scanning densitometry (Pharmacia LKB Biotechnology, Uppsala, Sweden), and the membranes were stripped with 0.1X SSPE (18 mmol/L NaCl, 1 mmol/L sodium phosphate, 0.1 mmol/L EDTA) containing 1 g SDS/L for 15 min at an initial temperature of 100°C and then 10 min at 25°C, then baked for 1 to 2 hr at 80°C before rehybridization with the next probe.

Statistics

Data are presented as means ± SEM. Results for each dietary Se treatment were subjected to one-way analysis of variance (ANOVA), and differences between means were assessed by Duncan's multiple range analysis.[26] Differences were considered statistically significant at the probability level $P < 0.05$.

Results

Growth

Rats were weighed throughout the treatment period to determine the effect of dietary Se supplementation on growth. Rats fed the Se-deficient basal diet that contained 0.007 μg Se/g diet, grew at the same rate as Se-supplemented rats (average 7.7 g/d) (Figure 1). Thus, the Se requirement for growth in weanling male rats is less than 0.007 μg Se/g diet under these conditions.

Liver selenium analysis

In rats fed the unsupplemented basal diet, liver Se concentration was 5 ± 1% of the level in rats fed 0.1 μg Se/g diet (*Figure 2A*), showing that the basal diet was Se-deficient. Supplementation with 0.02 μg Se/g did not significantly raise liver Se concentration above the unsupplemented levels, but liver Se increased sigmoidally with further increases in dietary Se, reaching a breakpoint at 0.1 μg Se/g diet (*Figure 2A*). The steepest increase in liver Se occurred between 0.05 and 0.1 μg Se/g. Above the breakpoint, supplementation with 0.15 μg Se/g diet did not significantly increase liver Se above 0.1 μg Se/g diet levels, but 0.2 μg Se/g diet produced a modest 15% increase when compared to rats fed 0.1 μg Se/g diet.

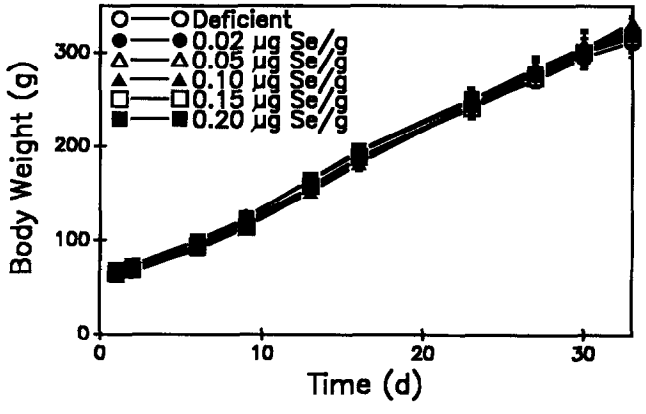

Figure 1 Growth in weanling male rats fed an Se-deficient diet (0.007 μg Se/g diet) supplemented with 0, 0.02, 0.05, 0.1, 0.15, or 0.2 μg Se/g diet as Na_2SeO_3 for 33 days. Male 21-day-old weanling rats weighing from 60 to 65 g were given free access to food and deionized water. Values are means ± SEM ($n = 3$ per group). There was no significant difference in rat weights at any time due to dietary treatment. The final body weights for the lightest (Se deficient) and heaviest (0.1 μg Se/g diet) groups at 33 days were 312 ± 16 g and 333 ± 18 g, respectively.

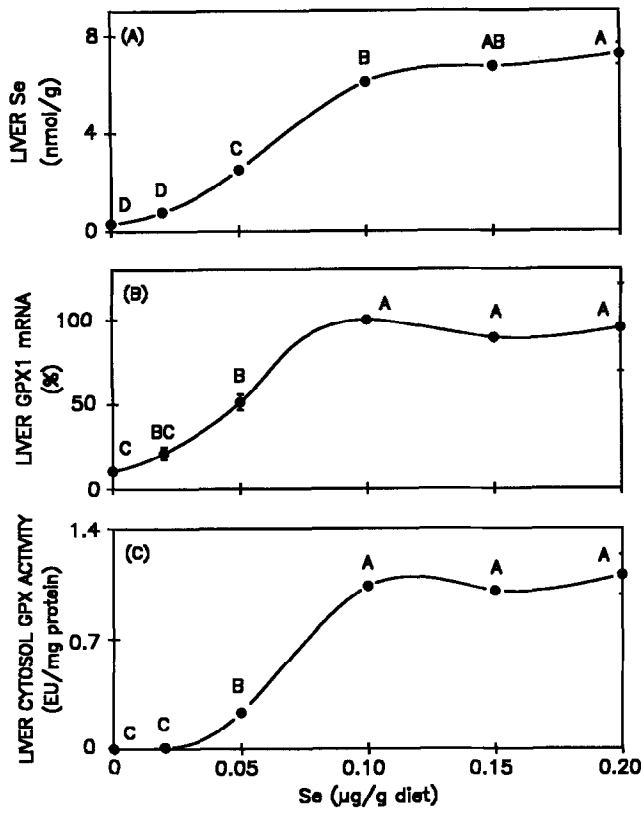

Figure 2 Effect of dietary Se concentration on liver Se concentration (A), liver glutathione peroxidase (GPX1) mRNA levels (B) and liver GPX activity (C). Weanling male rats were fed an Se-deficient diet (0.007 μg Se/g diet) with graded levels of supplemental Se for 33 days. (A) Liver Se concentration was determined by neutron activation analysis. (B) GPX1 mRNA levels were determined by Northern blot analysis using the GPX1 probe. GPX1 mRNA levels in rats fed 0.1 μg Se/g diet were set at 100%. (C) GPX activity was determined in liver cytosol using H_2O_2 as the substrate. Protein was determined by the method of Lowry et al. (1951). Values are means ± SEM ($n =$ 3 rats per group). For a given parameter, values not sharing a letter are significantly different ($P < 0.05$).

Plasma and erythrocyte GPX activity

Plasma GPX activity in rats fed the unsupplemented basal diet was 2 ± 1% of the levels in rats fed 0.1 μg Se/g diet (*Figure 3A*). With dietary Se supplementation, plasma GPX activity increased sigmoidally, with the steepest slope occurring between 0.02 μg Se/g diet and 0.05 μg Se/g diet (*Figure 3A*). The breakpoint for plasma GPX activity occurred before 0.1 μg Se/g diet. Further supplementation with 0.15 and 0.2 μg Se/g diet did not significantly raise plasma GPX activity above 0.1 μg Se/g diet levels.

Erythrocyte GPX activity was somewhat resistant to Se deficiency, with the unsupplemented levels remaining at 37 ± 1% of the levels in rats fed 0.1 μg Se/g diet (*Figure 3B*). Again, Se supplementation resulted in a sigmoidal response curve, with the steepest slope occurring between 0.05 and 0.1 μg Se/g diet (*Figure 3B*). An inflection point occurred

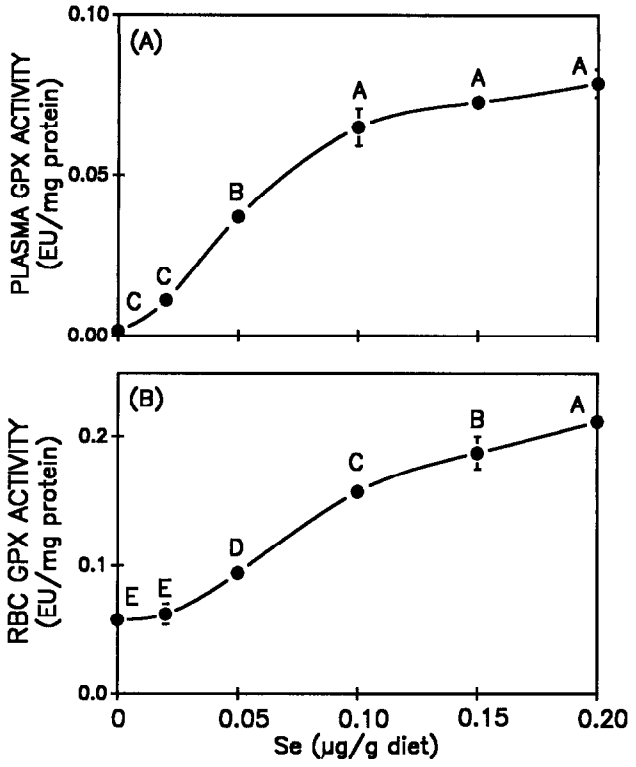

Figure 3 Effect of dietary Se concentration on plasma glutathione peroxidase (GPX) activity (A) and red blood cell (RBC) GPX activity (B). Weanling male rats were fed an Se-deficient diet (0.007 μg Se/g diet) with graded levels of supplemental Se for 33 days. GPX activity was determined using H_2O_2 as the substrate. Protein was determined by the method of Lowry et al. (1951). Values are means ± SEM (n = 3 rats per group). For a given parameter, values not sharing a letter are significantly different ($P < 0.05$).

at 0.1 μg Se/g diet for erythrocyte GPX and further supplementation with 0.15 and 0.2 μg Se/g diet produced more moderate, but statistically significant, increases in erythrocyte GPX activity levels.

Liver cytosol GPX activity

GPX activity was undetectable in liver cytosol from rats fed the Se-deficient basal diet (*Figure 2C*). Supplementation with 0.02 μg Se/g diet had virtually no effect on liver GPX activity when compared to the unsupplemented levels. With increasing dietary Se, liver GPX activity increased sigmoidally, reaching a plateau breakpoint at 0.1 μg Se/g diet (*Figure 2C*). Further increases in dietary Se above 0.1 μg Se/g diet had no further effect on liver GPX activity levels. Liver weight and liver protein concentration were not affected by any level of Se supplementation and there were no gross signs of liver necrosis (data not shown), as expected in animals supplemented with 100 mg vitamin E/kg diet. Thus, the loss of GPX activity in Se-deficient rat liver is not a secondary result due to poor health, but is rather a clear biochemical marker of Se deficiency.

GPX1 mRNA analysis

Northern blot hybridization using the murine GPX1 probe detected a single 13S GPX1 mRNA species in samples of total RNA isolated from liver (*Figure 4A*). Densitometry of Northern blot autoradiograms showed that GPX1 mRNA levels in Se-deficient rat liver were 10 ± 2% of levels in rats fed 0.1 μg Se/g diet. GPX1 mRNA levels increased sigmoidally with Se supplementation, and had reached plateau levels by 0.1 μg Se/g (*Figure 2B*).

To show that changes in GPX1 mRNA levels were the result of a Se-specific regulatory mechanism, poly A mRNA levels were determined for each sample by hybridization with a ^{35}S-labeled polythymidylate probe. This analysis

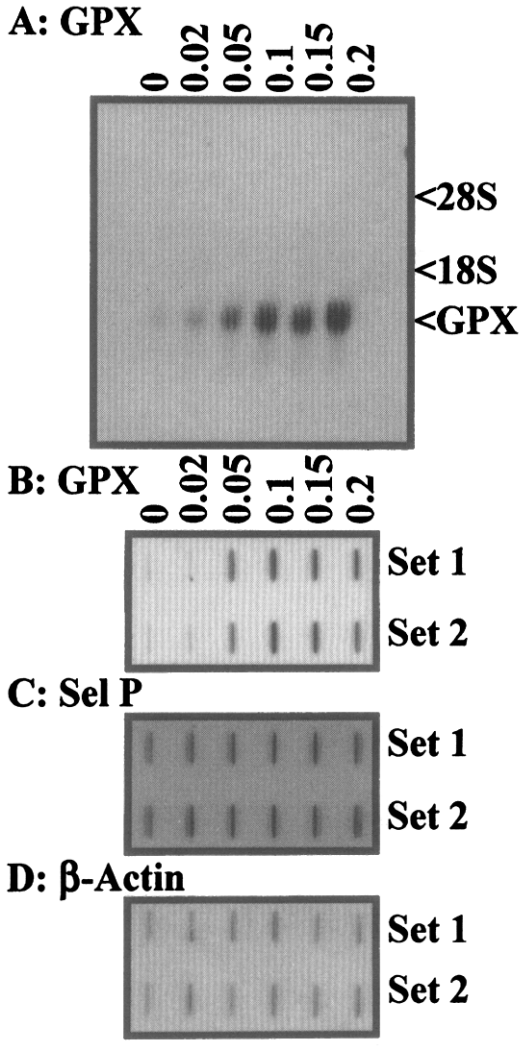

Figure 4 Representative Northern blot (A) and slot-blot autoradiograms (B,C,D) used to determine glutathione peroxidase (GPX1) mRNA, Sel P mRNA and β-actin mRNA levels in rats fed graded levels of dietary Se. Weanling male rats were fed an Se-deficient diet (0.007 μg Se/g diet) for 33 days and total RNA was isolated from liver tissue. For Northern blot analysis (A), 30 μg of total RNA per lane was separated by denaturing agarose gel electrophoresis. Arrows indicate the migration positions of 18S and 28S ribosomal RNA. RNA was transferred to membranes and hybridized with the ^{32}P-labeled probe for murine GPX1. For slot blot analysis (B), equal amounts of polyadenylated mRNA (approximately 7 μg of total RNA) were slot-blotted to a nylon membrane and hybridized with the ^{32}P-labeled probe for GPX1 mRNA. After autoradiography, the blot was stripped and rehybridized with the ^{32}P-labeled probe for Sel P mRNA (C). After autoradiography, the blot was stripped and rehybridized with the ^{32}P-labeled probe for β-actin mRNA (D).

showed that Se status had no effect on poly A mRNA levels in the rat liver (*Table 1*). As expected, when samples that contained equal amounts of poly A mRNA were subjected to slot-blot analysis using the GPX1 probe (*Figure 4B*), densitometric analysis showed results analogous to those obtained by Northern blot analysis. Se deficiency decreased liver GPX1 mRNA level to 15% of Se-adequate levels. Thus, Se regulation of GPX1 mRNA level is dramatic, whether expressed relative to total RNA levels or relative to poly A mRNA levels.

Slot blots were re-probed to quantitate the mRNA level for a second selenoprotein, selenoprotein P (Sel P) (*Figure 4C*). Densitometry of the autoradiograms showed Sel P mRNA levels in the unsupplemented rats were 69 ± 2% of the levels in rats fed 0.1 μg Se/g diet (*Table 1*), but the differences in Sel P mRNA levels between Se treatments were not statistically significant.

As a control, the slot blots were re-probed for β-actin mRNA (*Figure 4D*). Densitometry of the autoradiograms showed that β-actin mRNA levels in liver samples were not affected by Se status.

Discussion

We have determined liver GPX1 mRNA level and six other Se-dependent parameters in the same young male rats to critically evaluate the ability of these parameters to indicate dietary Se status. Numerous studies have determined that 0.1 μg Se/g diet is required for maximal liver GPX activity[3,27] and the present study also affirms that 0.1 μg Se/g diet is required for liver GPX activity to plateau in rapidly growing male rats. The characteristic plateau in liver GPX activity indicates that the Se-dependent component of the mechanism for GPX synthesis becomes saturated when dietary Se reaches 0.1 μg Se/g diet. This clear breakpoint for liver GPX activity provides a particularly useful point of reference for further understanding the relationship of other parameters to Se status.

Plasma and erythrocyte GPX activities are frequently used as convenient parameters for assessing Se status.[28–30] In other studies with male and female rats, we observed that plasma GPX activity has a plateau breakpoint at 0.07 μg Se/g diet.[6,24] Graphical analysis in the present study also indicates that the plateau breakpoint for male rat plasma GPX activity occurs before 0.1 μg Se/g diet. Male rat erythrocyte GPX activity levels showed an inflection point at 0.1 μg Se/g diet. The common breakpoints in Se metabolism in both erythrocyte and liver parameters strongly indicates that 0.1 μg Se/g diet is potentially a meaningful point at which to set the dietary Se requirement for the rapidly growing male rat. Notably, erythrocyte GPX activity continued to increase significantly with increasing dietary Se in the present study, and previous studies have shown that erythrocyte GPX activity continues to increase to near toxic levels of dietary Se.[1] The lack of a clear plateau can curtail the usefulness of erythrocyte GPX as an indicator of Se status. For example, an increase in erythrocyte GPX activity after Se supplementation does not necessarily indicate that the original diet was deficient in Se.

Similarly, 0.1 μg Se/g diet did not result in maximal liver Se concentration in the present study, although a clear inflection point was again present at 0.1 μg Se/g diet for this parameter. The fact that liver Se concentration does not continue to rise dramatically beyond 0.1 μg Se/g diet suggests that Se detoxification and excretion pathways can compensate substantially for further modest increases in dietary Se. Thus, the plateau breakpoint for GPX activity and the concurrent inflection in liver Se concentration may mark the beginning of excessive dietary Se. If true, this would mean that a dietary Se requirement based on maximal tissue Se retention is likely an overestimate, resulting in the need for Se excretion. The interpretation of tissue Se concentration as an indicator of Se status is further complicated because this parameter does not distinguish between different forms of Se or different pools of Se. Thus, near and beyond the Se requirement, tissue Se concentration might include Se in forms that do not serve an important biologic function.

We hypothesize that GPX1 mRNA level is one parameter that directly reflects the level of an important metabolic pool of Se. In the present study, Se-deficient GPX1 mRNA levels were 10 to 15% of Se-adequate levels, similar to previous reports.[14,16,17] This 85 to 90% decrease in GPX1 mRNA level was observed using Northern blot analysis for samples with equal amounts of total RNA and by slot-blot

Table 1 Relative poly A mRNA levels, GPX1 mRNA levels, and selenoprotein P mRNA levels in male rat liver[1]

	Dietary Se (μg/g diet)[2]					
	0	0.02	0.05	0.1	0.15	0.2
mRNA			%			
polyA[3]	107 ± 14^{A}	110 ± 13^{A}	109 ± 9^{A}	100 ± 14^{A}	99 ± 14^{A}	127 ± 5^{A}
GPX1[4]	15 ± 1^{D}	21 ± 2^{D}	53 ± 4^{C}	100 ± 0^{A}	83 ± 11^{AB}	74 ± 12^{B}
SelP[5]	69 ± 2^{A}	87 ± 13^{A}	95 ± 9^{A}	100 ± 8^{A}	98 ± 9^{A}	99 ± 12^{A}

[1]Values are relative means ± SEM (n = 3). The mean values for rats fed 0.1 μg Se/g diet were set at 100% for each row. Within a row, values with different superscripts are significantly different ($P < 0.05$).
[2]Rats were fed an Se-deficient basal diet (0.007 μg Se/g diet) with graded levels of supplemental Se as Na_2SeO_3.
[3]Relative levels of poly A mRNA were determined by slot-blot analysis using the ^{35}S-labeled polythymidylate probe.
[4]GPX1 mRNA levels were determined by slot-blot analysis of total RNA samples that contained equal amounts of poly A mRNA.
[5]Sel P mRNA levels were determined by slot-blot analysis of total RNA samples that contained equal amounts of poly A mRNA.

analysis for samples with equal amounts of mRNA. Dietary Se status had no effect on poly A mRNA levels or on β-actin mRNA levels, further demonstrating that the mechanism for Se regulation of GPX1 mRNA is specific. The order-of-magnitude change in liver GPX1 mRNA level from deficient to adequate Se status makes this parameter especially useful in understanding Se requirements.

In addition to GPX1, we determined mRNA levels for a second selenoprotein, plasma Sel P. In the present study, Se-deficient levels of selenoprotein P mRNA decreased insignificantly to 69% of Se-adequate levels. Other studies have also shown that mRNA levels for Sel P,[16] type I 5'-deiodonase,[7] and phospholipid hydroperoxide glutathione peroxidase[6] can show moderate decreases during Se deficiency, suggesting that when selenocysteine is not readily available during translation, there is a moderate, general decrease in mRNA for all selenoproteins. The rapid and dramatic decrease that is consistently evident for GPX1 mRNA levels during Se deficiency suggests that the mechanism for Se regulation of GPX1 mRNA has a unique facet when compared to that of other selenoproteins.

The relationship between Se incorporation during translation and Se regulation of GPX1 mRNA levels remains a compelling question. Graphical analysis of GPX activity versus GPX1 mRNA levels shows that with incremental increases in dietary Se, relative increases in GPX1 mRNA levels precede the changes in GPX activity (*Figure 5*). This shows that Se regulation of GPX activity is not simply due to a limitation of available selenocysteine during translation. Half-maximal GPX1 mRNA levels occurred before 0.05 μg Se/g diet whereas half-maximal GPX activity levels occurred at 0.07 μg Se/g diet. In other studies with rats[6,24] and with cultured cells[20] we have shown that GPX1 mRNA levels clearly reach the plateau breakpoint at a lower Se level than for GPX activity. In the present study, we needed an intermediate dietary treatment between 0.05 and 0.1 μg Se/g diet to show more conclusively that GPX1 mRNA levels reach the plateau breakpoint before GPX activity. As shown in *Figure 5*, the relationship between GPX activity and GPX1 mRNA levels breaks down above 0.1 μg Se/g diet, demonstrating again that above this regulatory breakpoint, neither GPX1 mRNA nor GPX activity in liver are affected by further increases in Se.

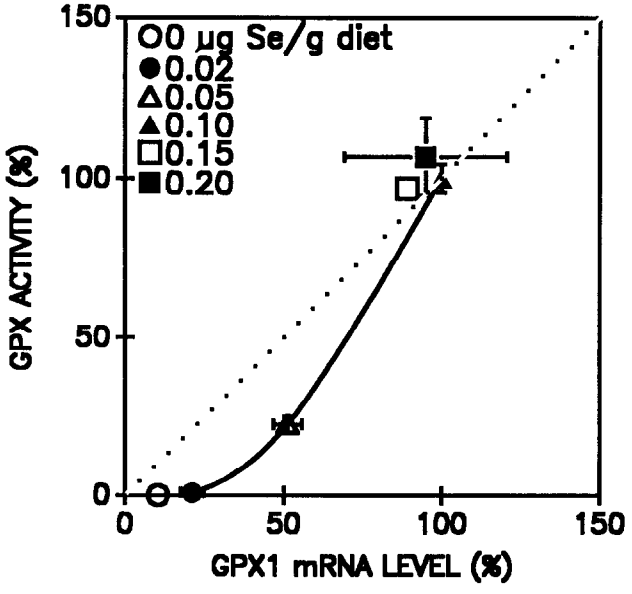

Figure 5 Effect of dietary Se concentration on the relationship between GPX1 mRNA levels and GPX activity levels. Weanling male rats were fed an Se-deficient diet (0.007 μg Se/g diet) for 33 days. Relative liver GPX activity levels were plotted versus relative liver GPX1 mRNA levels at each dietary Se level. Values are means ± SEM (n = 3 rats per group).

We hypothesize that the saturation of GPX1 mRNA level by Se depends on the affinity of an as yet unidentified Se-binding protein that interacts both with the GPX1 mRNA and with a metabolic pool of Se that is drawn upon for the synthesis of GPX1 and other selenoproteins. This metabolic Se pool might be selenocysteine esterified to the unique $tRNA^{Sec\ 31}$ or the reactive Se donor, selenophosphate, used in the formation of the $tRNA^{Sec}$.[32,33] Thus, GPX1 mRNA level may specifically reflect this critical intracellular Se pool, and would not be directly influenced by Se which is already incorporated into proteins, either as selenocysteine or selenomethionine. Previous studies have shown that high levels of dietary selenomethionine can yield low GPX activity, illustrating this relationship.[34] In Se-deficient thyroid, GPX activity drops to half of Se-adequate levels but GPX1 mRNA is unchanged[7] indicating that the available Se pool in this tissue does not drop sufficiently to down-regulate thyroid GPX1 mRNA.

Until the molecular mechanism for Se regulation of GPX1 mRNA is more completely understood, GPX1 mRNA level may be the most direct and most sensitive parameter currently available to measure metabolically active Se. Se regulation of GPX1 mRNA is potentially linked to a cellular "Se thermostat" that enables each cell to sense and respond to daily changes in dietary Se. Down-regulation of GPX1 mRNA during Se-deficiency would allow critical Se to be preferentially incorporated into selenoproteins encoded by mRNA that is relatively unaffected by Se deficiency. When dietary Se exceeds Se flux into these critical selenoproteins, intracellular free Se would up-regulate GPX1 mRNA levels, resulting in an increase in GPX1 synthesis. This, in turn, would dampen the increase in the intracellular Se pool. The sharp rises in liver Se, liver GPX1 mRNA, and liver GPX activity (*Figure 3*) show this response clearly, and *Figure 5* shows that the relative change in GPX1 mRNA precedes the change in GPX1 activity. Additional increases in GPX1-Se might serve as a reserve for future Se needs during times of low dietary Se intake.[27] We hypothesize that the plateau breakpoint for GPX1 mRNA represents saturation of an intracellular free Se pool, whereas the plateau breakpoint for GPX activity represents maximization of intracellular Se storage. Thus, dietary Se levels that maximize GPX activity may already include a built-in evolutionarily-derived safety factor.

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