Dietary Selenium Requirements Based on Glutathione Peroxidase-1 Activity and mRNA Levels and Other Se-Dependent Parameters Are Not Increased by Pregnancy and Lactation in Rats

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ABSTRACT The hierarchy of selenium (Se) requirements for growing rats ranges from <0.01 to 0.1 μg Se/g diet, depending on the choice of Se status parameter. To further evaluate the efficacy of molecular biology markers to determine Se requirements in later periods of the life cycle, which are less amenable to traditional approaches, we studied pregnant and lactating rats. Female weanling rats were fed a Se-deficient diet (<0.01 μg Se/g) or supplemented with graded levels of dietary Se (0–0.3 μg Se/g) for >10 wk, bred, and killed on d 1, 12, and 18 of pregnancy and d 7 and 18 of lactation; Se response curves were determined for 10 parameters including liver glutathione peroxidase (GPX). Growth, and mRNA levels for selenoprotein P, 5'-deiodinase, and GPX4 were not decreased by Se deficiency. GPX4 activity required 0.05 μg Se/g diet for maximum activity, similar to growing rats. Dietary Se requirements for plasma GPX3 activity decreased 33% in pregnancy, but returned during lactation to the requirement of growing rats. The Se requirement for GPX1 activity decreased 25% in pregnancy but not in lactation. GPX1 mRNA required 0.05 μg Se/g diet for maximum levels in both pregnancy and lactation, similar to growing rats. Clearly, Se requirements do not increase during pregnancy and lactation relative to Se requirements in growing rats. Unexpectedly, Se-adequate levels of GPX1 mRNA and activity declined to <40 and 50%, respectively, of nonpregnant Se-adequate levels during pregnancy and lactation, illustrating the need to fully understand biomarkers at all stages of the life cycle.

KEY WORDS: biomarkers • gene expression • molecular biology • RDA • ribonuclease protection

The laboratory rat has been an indispensable tool and model of choice in the study of nutrition as well as in experimental medicine (1). In nutrition, dietary selenium (Se) requirements and rodent experiments have been intimately associated starting with the discovery of the essentiality of Se; Schwarz and Foltz (2) found that 0.04 μg Se/g diet in a Se-deficient diet would prevent liver necrosis in weanling rats. Hurt et al. (3) reported that 0.05 μg Se/g diet prevents growth depression in rats, but more recent studies indicate that as little as 0.02 μg Se/g diet is required to sustain growth in pups from Se-deficient dams (4). In rats fed selenite, 0.1 μg Se/g diet is the minimum level required to reach plateau levels of liver Se (5,6). With the discovery that glutathione peroxidase (GPX)5 was a Se-dependent enzyme, Hafemann et al. (7) found that 0.1 μg Se/g diet was sufficient to maximize activity of this biochemical marker in RBC and liver, and numerous additional studies affirmed that 0.1 μg Se/g diet is the dietary Se requirement based on these biochemical parameters. As additional selenoproteins were identified, these new biochemical markers were also used to assess Se requirements in rapidly growing young rats (Table 1).

The discovery that the levels of GPX1 mRNA are also highly regulated by Se status (8) led to a series of studies showing that GPX1 mRNA could be used as a molecular biology marker to evaluate requirements. In these experiments, use of a basal Se-deficient diet (<0.01 μg Se/g) and 5 or more graded levels of Se supplementation resulted in sigmoidal or hyperbolic selenium response curves that can be used to graphically determine the minimum dietary Se required for maximal levels of each measured parameter (5). In liver of male and female rats, liver GPX1 activity falls essentially to zero in rats fed a typical Se-deficient diet, and rises sigmoidally with increasing dietary Se to reach a plateau in the Se response curve at 0.1 μg Se/g diet (5,6). This regulation of GPX1 activity is accompanied by regulation of GPX1 mRNA.

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3 Supplemental Tables 1–11 are available as Online Supporting Material with the online posting of this paper at www.nutrition.org.
4 To whom correspondence should be addressed.
5 Abbreviations used: 5DI, thyroxine 5'-deiodinase; EU, enzyme unit; GPX, glutathione peroxidase; RPA, ribonuclease protection assay; SelP, selenoprotein-P.
6 GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPX, glutathione peroxidase; RPA, ribonuclease protection assay; SelP, selenoprotein-P.
which falls to as low as 6–10% of plateau levels and rises in a Se response curve to a plateau level at ~0.05 μg Se/g diet (5).

Thus, selenoprotein mRNA levels have also been used as biomarkers to determine dietary Se requirements in rats. The result is a hierarchy of dietary Se requirements for growing rats ranging from the indicated parameter to reach plateau levels when Se-adequate weanling rats are fed these diets from weaning.

The current Se requirement (13) for pregnant/lactating rats is not increased above the minimal Se requirement of 0.1 μg Se/g diet (13) for growing rats thus meets all of the minimum requirements listed in Table 1, allows for variation, and is even sufficient to prevent microvascular damage in the retinai of rats fed high-sucrose diets (14).

The current Se requirement (13) for pregnant/lactating rats states that the minimum requirement during pregnancy and lactation is 0.4 μg Se/g diet, and thus much higher than for young rapidly growing rats. The growth rate of female rats, however, slows considerably after 50 d of age (4), and heat production and food intake are increased in pregnant and lactating rats relative to nonpregnant female rats (13), such that Se needed for fetal growth and lactation would be provided by increased food intake. In addition, homeostatic mechanisms are likely to facilitate increased retention of Se if needed. Collectively, this suggests that the current estimate for the dietary Se requirement in pregnant and lactating rats is high.

To further evaluate the efficacy of using molecular biology markers to determine the Se requirements in later periods of the life cycle, which are less amenable to traditional approaches, we studied pregnant and lactating rats. Our hypothesis was that the Se requirement during pregnancy or lactation does not increase above the minimal Se requirement of 0.1 μg Se/g diet for young rapidly growing rats. We found that the minimal requirement for maximal expression of GPX1 activity actually decreases in pregnant rat stages to younger rats. In addition, we found a significant downregulation of both maximal GPX1 activity and maximal GPX1 mRNA level during lactation in Se-adequate rats.

### TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Growing rats</th>
<th>Pregnancy</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver SelP mRNA</td>
<td>&lt;0.01 (6)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Liver GPX4 mRNA</td>
<td>&lt;0.01 (9)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Rat growth</td>
<td>&lt;0.01 (5)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Liver necrosis</td>
<td>0.04 (2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Liver GPX4 activity</td>
<td>0.05 (8)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Liver GPX1 mRNA</td>
<td>0.05 (5)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Liver TRR1 mRNA</td>
<td>0.05 (10)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Liver 5DI mRNA</td>
<td>0.05 (11)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma GPX3 activity</td>
<td>0.075 (7)</td>
<td>0.05</td>
<td>0.075–0.1</td>
</tr>
<tr>
<td>Liver TRR1 activity</td>
<td>0.075 (10)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RBC GPX1 activity</td>
<td>0.1 (7)</td>
<td>0.05</td>
<td>0.05</td>
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<tr>
<td>Liver GPX1 activity</td>
<td>0.1 (5)</td>
<td>0.05–0.075</td>
<td>0.075–0.1</td>
</tr>
<tr>
<td>Liver Se</td>
<td>0.1 (5)</td>
<td>0.075</td>
<td>0.1</td>
</tr>
<tr>
<td>Liver 5DI activity</td>
<td>0.1 (11)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Plasma SelP</td>
<td>0.1 (12)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Growing rat dietary Se requirements from references cited in parentheses. Requirements are the minimum dietary Se necessary for the indicated parameter to reach plateau levels when Se-adequate weanling rats are fed these diets from weaning.**

**Dietary Se requirements for pregnant and lactating rats are from the present study. TRR1, thioredoxin reductase-1.**
GPX1, GPX4, and SelP mRNA signals were normalized to the GAPDH mRNA signal.

Liver and dietary Se concentrations were determined by neutron activation analysis (20).

Data are presented as means ± SEM, n = 3/group at each stage of pregnancy; at d 18 of lactation, groups at 2 levels of Se supplementation had <3 rats per group (21 total for 8 groups); at d 7 of lactation, groups at 4 levels of Se supplementation had <3 rats per group (18 total for 8 groups). At each time point, dietary treatment groups were subjected to 1-way ANOVA, and differences between means were assessed by Duncan’s multiple range analysis (P < 0.05), with Kramer’s modification for unequal class sizes where necessary (21). Detailed statistical analysis is provided in Supplemental Tables 1–113. The plateau breakpoint for each Se response curve, defined as the intersection of the line tangent to the point of steepest slope and the plateau, was calculated as described by Weiss et al. (5,6) using polynomial regression analysis (Sigma Plot, Jandel Scientific) to estimate the minimum dietary Se necessary to obtain plateau responses.

RESULTS

Dietary Se did not affect weight at any stage of pregnancy or lactation (Fig. 1). At d 1 of pregnancy, the Se-deficient rats weighed 262 ± 7 g; weights of Se-supplemented rats ranged from 264 ± 8 g for the 0.3 μg Se/g group to 277 ± 10 g for rats fed 0.05 μg Se/g; on the same day, nonpregnant female rats that were not hormone-treated weighed 256 ± 21 g. The mean increase in body weight over the course of pregnancy from d 1 to 18 was 101.5 g and there were no significant differences among dietary treatments, with the smallest increase occurring in rats administered 0.075 μg Se/g, and the largest increase (113 g/d) in Se-deficient rats. Thus, in this study, Se deficiency and dietary Se status did not affect weight gain during pregnancy and lactation.

At d 1 of pregnancy, liver Se concentrations in rats fed the deficient diet were 5% of levels in rats fed 0.2 μg Se/g diet (Fig. 2A). With increasing dietary supplementation, there was a hyperbolic response with the plateau breakpoint at 0.075 μg Se/g such that rats fed diets containing 0.075 μg Se/g did not differ from rats fed higher levels of dietary Se. At d 12 and 18 of pregnancy, the response curves were similar to d 1. Statistically similar response curves were observed at d 7 and 18 of lactation, but graphical analysis showed that the breakpoint had shifted back to 0.1 μg Se/g diet in lactating rats, indicating that there was a shift in the requirement for maximal liver Se concentration from 0.075 μg Se/g diet during pregnancy back to 0.1 μg Se/g diet during lactation.

Plasma GPX3 activity in nonpregnant female rats was 17% higher than that observed in Se-adequate (0.2 μg Se/g diet) pregnant rats on d 1 of pregnancy (Fig. 2B). At d 1 of pregnancy, Se-deficient rats had plasma GPX3 activities that
were 14% of Se-adequate rats. With increasing dietary Se, there was a hyperbolic response to Se such that rats fed 0.05 μg Se/g torula yeast did not differ from rats fed higher levels of Se. Similar results were observed at d 12 and 18 of pregnancy. In Se-deficient lactating rats, however, plasma GPX3 activities were 2–4% of Se-adequate values and lower than in pregnant rats. The Se-adequate values were similar to those of nonpregnant female rats; the apparent plateau breakpoints were between 0.075 and 0.1 μg Se/g diet, thus differing little from weanling rats (5).

RBC GPX1 activity in Se-adequate nonpregnant rats was similar to Se-adequate pregnant and lactating rats (Fig. 2C). GPX1 activity in Se-deficient RBC ranged between 16 and 19% of Se-adequate levels; the response curves were basically superimposable, with maximal increases in GPX1 activity occurring between 0 and 0.05 μg Se/g diet, and the plateau breakpoints clustered around 0.05 μg Se/g diet.

In liver, GPX1 activity was 33% higher in nonpregnant females compared with d 1 pregnant females fed 0.2 μg Se/g diet (Fig. 2D). In rats fed the Se-deficient diet, GPX1 activity was 2–4% of Se-adequate levels at all stages of pregnancy and lactation. Graded levels of dietary Se resulted in sigmoidal response curves as noted previously (5). In pregnancy, the most rapid increases in liver GPX1 were found between 0.02 and 0.05 μg Se/g. Values at 0.075 μg Se/g were not significantly different from rats fed higher levels of Se in pregnancy with the plateau breakpoint occurring between 0.05 and 0.075 μg Se/g diet. In contrast, Se-adequate female rats at d 7 and 18 of lactation had <70% of the liver GPX1 activity at d 1 of pregnancy and ~50% of the levels in the nonpregnant Se-adequate rats. GPX1 activity was not affected when the dietary Se level was increased from 0.2 to 0.3 μg Se or decreased to 0.1 or 0.15 μg Se/g diet, clearly indicating that this drop in plateau liver GPX1 activity was not due to available dietary Se. The response of liver GPX1 activity to dietary Se was also sigmoidal in lactating rats, with the most rapid increase occurring between 0.02 and 0.075 μg Se/g but the plateau was not reached until 0.1 μg Se/g diet in d 7 lactating rats.

Liver GPX4 activity in nonpregnant Se-adequate rats was similar to the activities in pregnant and lactating rats fed Se-adequate diets (Fig. 2E). In Se deficiency, liver GPX4 activity fell to between 20 and 37% of Se-adequate levels with the rapid rise in activity occurring between 0 and 0.05 μg Se/g diet. By 0.05 μg Se/g diet, the plateau had been reached at all stages of pregnancy and lactation.

In Se-adequate rats, RPA readily detected protected fragments for GPX1, GPX4, SelP, and 5DI mRNA, as well as GAPDH (Fig. 3). In Se-deficient pregnant female rats at d 1 or 18, liver GPX1 mRNA levels fell dramatically relative to Se-adequate rats. At d 18 of lactation, Se-deficient rats also had decreased levels of GPX1 mRNA, but the levels of GPX1 mRNA in Se-supplemented rats in lactation were clearly lower than at d 18 of pregnancy, and that level at d 18 of pregnancy was less than seen on d 1 of pregnancy. In Se-deficient rats, GPX4 mRNA levels were similar to Se-supplemented levels; mRNA levels for the other selenoproteins did not appear to vary dramatically.

Quantitation of liver GPX1 mRNA expression (Fig. 4) by instant imaging revealed that Se-deficient d 1 pregnant rats had 20% of the levels in nonpregnant Se-adequate rats. Se-deficient d 18 pregnant rats had 13% and d 18 lactating rats had <10% of the levels of GPX1 mRNA in Se-adequate rats. There was a hyperbolic response in GPX1 mRNA level with increasing Se supplementation at each stage of pregnancy or lactation, with the plateau reached at 0.05 μg Se/g at each stage. When Se-adequate (0.2 μg Se/g) mRNA levels in pregnancy and lactation were compared directly with the levels found in nonpregnant Se-adequate rats (Fig. 5), there was a gradual decline in GPX1 mRNA during pregnancy and lactation such that d 18 lactating rats had <40% of the levels in nonpregnant rats. Notably, this decrease in mRNA was not abolished by additional dietary Se (0.3 μg Se/g). Levels of GPX4 mRNA were reduced relative to levels found in nonpregnant female rats (Fig. 4B) but were not significantly different during pregnancy and lactation in Se-adequate rats (Fig. 5). This was also the case for SelP mRNA and GAPDH mRNA levels (Fig. 4C, E), clearly indicating that the reduction in GPX1 mRNA level during pregnancy and lactation was distinct relative to the lack of effect of Se status on these other selenoprotein mRNAs. In lactating rats, however, 5DI mRNA levels were 30–40% of levels in nonpregnant rats, but unlike GPX1 mRNA, Se status did not significantly affect 5DI mRNA levels during lactation.

DISCUSSION

Immediately after the discovery that Se was essential for GPX1 enzymatic activity, selenium researchers began using biochemical tools to establish Se requirements. Overall, in these studies using weanling animals and under conditions of rapid growth, ~0.1 μg Se/g was the minimum level of dietary Se that would allow attainment of the plateau or maximal level of GPX1 activity (Table 1). Expansion of Se requirement determinations to more difficult situations, such as adult animals, animals that are pregnant and lactating, or elderly animals, is far more problematic both because of variations in initial feeding periods and tissue stores as stored nutrient will modulate requirements, and because adult animals may be growing at proportionally different rates and thus have diminished true needs beyond that due to regular turnover.

The efficacy of GPX1 activity for assessing requirements is apparently enhanced because the underlying GPX1 mRNA is also regulated highly by Se status and can be used to assess Se requirements (Table 1). In this study, we extended the use of mRNA for assessing nutrient requirements to evaluate nutri-
ent requirements of pregnant and lactating rats because the 1995 NRC report (13) suggests that the dietary Se requirement in rats is dramatically increased during pregnancy and lactation. The result was a comprehensive study in which nutrient requirements were evaluated at 5 stages of pregnancy and lactation (Table 1).

In these adult female rats, which had been fed the experimental levels of dietary Se from weaning and for 10 wk, we did not identify any effect of dietary Se on body weight, clearly indicating that the basal level of Se, 0.01 g Se/g diet, is fully adequate for maintaining growth in these rats with presumably normal Se stores as purchased. Similarly, the rats gained 100 g over 17 d of pregnancy and, again, there was no effect of Se status on the growth of these rats, indicating that 0.01 g Se/g diet is adequate for growth during pregnancy. There was also no effect of Se status on pregnancy outcome or litter size (data not shown). It may be that earlier reports of detrimental effects of Se deficiency on reproduction occurred because rats were Se deficient (22) at the start of the experiment and/or because other dietary components such as vitamin E or the sulfur amino acids may have been more limiting in those studies than in the present study. Similarly, there were no effects on weight gain during lactation due to differences in

FIGURE 4  Effect of dietary Se supplementation on selenoprotein mRNA levels at different stages of pregnancy and lactation in rats. Rats were fed graded levels of dietary Se and analyzed at d 1 and 18 of pregnancy, and d 18 of lactation for each selenoprotein mRNA. RPA autoradiographs were analyzed by direct imaging, and the values for each mRNA are expressed relative to the mean values for Se-adequate nonpregnant rats. Values represent the means ± SEM. For GPX1 mRNA, at each stage of pregnancy and at d 18 of lactation, means for Se-deficient rats were significantly different (P < 0.05) from means from rats fed >0.05 µg Se/g diet. For Sel P and 5DI mRNA, dietary Se supplementation did not affect any stage of pregnancy or lactation. Dietary Se supplementation did not affect GPX4 mRNA at d 1 or 18 of pregnancy or GAPDH mRNA at d 1 of pregnancy. Detailed statistical analysis is provided in Supplemental Tables 7–11.

FIGURE 5  RPA (A) and quantitation (B) of selenoprotein mRNA expression in Se-adequate (0.2 µg Se/g diet) rat liver. Rats were killed at d 1 (P1), 12 (P12), 18 (P18) of pregnancy and d 7 (L7) and 18 (L18) of lactation, and analyzed for GPX1, GPX4, GAPDH, and Sel P, and on a second gel for 5DI. Also shown are nonpregnant (N-P) females. (A) Representative RPA. (B) Bars represent means ± SEM, n = 3, expressed as a percentage of the value for nonpregnant females. Means not sharing a letter differ, P < 0.05.
dietary Se, and at d 18 of lactation, the pup weights were not affected by dietary Se (data not shown). Thus, in this comprehensive set of studies, the Se requirement for sustaining growth, reproduction, pregnancy, and lactation was <0.01 μg Se/g diet for all these phases. It is important to remember that these results are distinct from other recent studies using 2nd-generation Se-deficient rats in which there are clear and large effects of Se-deficient diets on growth within the first 14 d after weaning (4).

In rapidly growing female rats fed the Se-deficient torula yeast diet, liver Se stores fell to 4% of plateau levels and the plateau in the liver Se response curve was reached at 0.1 μg Se/g diet (5). In the present study, the response curves for lactating rats were similar such that the plateau was achieved by 0.1 μg Se/g diet; in pregnant rats, however, the response curves were shifted to the left, showing that the Se requirement based on liver Se in pregnant rats decreases during pregnancy by 0.025 μg Se/g or 25%. We suggested previously that liver GPX1 in rats serves as a Se buffer or store that provides Se during times of greater dietary need (23,24); in these adult pregnant female rats, these data indicate that a reduced concentration of dietary Se is required to maintain liver Se stores at plateau levels.

In our previous study, plasma GPX3 activity reached a plateau breakpoint at ~0.075 μg Se/g (5), and the response curves in the present study during both pregnancy and lactation are similar, indicating that the dietary requirement for maintaining plasma GPX3 in young adult pregnant and lactating rats is also between 0.05 and 0.1 μg Se/g. RBC breakpoints in the response curves in these experiments were clearly lower than those observed previously in weanling rats, with the plateau breakpoint at 0.05 μg Se/g for RBC GPX1 vs. 0.1 μg Se/g in weanling rats (5). One reason for this difference may be that the lifespan of the RBC in rats is ~60 d; thus, the full magnitude of the RBC response could be detected in this 11- to 17-wk study, whereas the previous study with weanling rats examined changes after only 4 wk of dietary treatment. Using these blood parameters, the Se requirement did not increase in pregnancy and lactation based on plasma GPX3 activity and actually decreased 50% based on RBC GPX1 activity (Fig. 2B, C).

Liver GPX1 activity levels in these studies are very interesting. In pregnant rats, we observed sigmoidal curves of GPX1 activity relative to dietary Se levels, but the plateau breakpoint shifted in the pregnant rats from the 0.1 μg Se/g observed in weanling female rats (5) to 0.05 μg Se/g at d 1 of pregnancy and to 0.075 μg Se/g at d 12 and 18 of pregnancy. Additional dietary Se at 0.2 or 0.3 Se/g did not significantly increase the level of liver GPX1 activity, clearly indicating that the requirement based on liver GPX1 activity in pregnancy is reduced by 25–50% relative to the requirement for rapidly growing female rats. More startling was the response in lactating rats. The Se response curves shifted back and were similar to those observed in rapidly growing female rats, with a plateau breakpoint at ~0.1 μg Se/g, but the maximal level of GPX1 activity was 50% of nonpregnant females and 40% lower than that observed in the pregnant rats. Additional Se at 0.2 or 0.3 μg Se/g in the diet did not raise the liver GPX1 activity in these rats above levels at 0.1 μg Se/g. Thus the Se requirement during pregnancy based on liver GPX1 activity decreased at least 25% relative to that in rapidly growing weanling rats, but during lactation the Se requirement returned to the 0.1 μg Se/g diet value observed in rapidly growing weanling rats. Se response curves for liver GPX1 activity and liver Se in the pups at d 18 of lactation also had breakpoints at 0.1 μg Se/g diet (data not shown), providing additional support for a lack of an increase in the Se requirement in pregnant and lactating rats above that of young growing rats. The apparent 40% drop in maximal liver GPX1 activity in Se-adequate lactating rats (Fig. 2D) clearly shows that this biomarker has to be well understood before it can be used in isolation for estimating nutrient requirements.

In contrast to GPX1 activity, GPX4 activity response curves in pregnancy look very similar to those found in male weanling rats (9). The regulation of liver GPX4 activity by dietary Se in female weanling rats has not been studied, however, so it is not clear whether GPX4 activity is altered by pregnancy.

We used ribonucleic acid protection analysis to evaluate the levels of liver selenoprotein mRNAs in these studies. As reported previously, Se status has a negligible effect on GPX4 mRNA, SelP mRNA, 5DI mRNA, as well as GAPDH mRNA levels in rat liver (5,19). At each stage of pregnancy and lactation, GPX1 mRNA response curves had breakpoints at 0.05 μg Se/g, similar to those in weanling rats and indicating that this Se requirement was not affected by stage of pregnancy and lactation. What was dramatically altered, however, was that during pregnancy and lactation, the Se-adequate plateau levels of GPX1 mRNA dropped sequentially from d 1 through d 18 of pregnancy and through d 7 and 18 of lactation (Fig. 5). Higher dietary Se levels at 0.2 and 0.3 μg/g had no effect on the mRNA levels, indicating that Se was not the limiting factor. Instead, changes during pregnancy and lactation, perhaps hormonal, apparently downregulate transcriptional control of the mRNA. The biological effect of this natural reduction in GPX1 expression may be that this releases Se stored in rat liver so that it can be used to maintain necessary functions throughout the body and to provide Se for fetal growth and lactation. In addition to GPX1 mRNA expression, the mRNA levels for GPX4, SelP, and 5DI also decreased but more modestly than GPX1 mRNA during pregnancy and lactation irrespective of dietary Se level (Fig. 4). Although not measured here, the levels of Se excretion and deposition of Se in fetus and pup would be helpful to fully understand the flux of Se during pregnancy and lactation.

The Se requirement of 0.4 μg Se/g diet suggested by the NRC (13) for pregnant and lactating rats is based on a study using casein-based diets containing 0.025, 0.05, 0.1, or 0.2 μg Se/g diet (25). That study started with nulliparous female rats weighing ~170 g that were fed diets for only 2–3 wk. The report found that RBC GPX1 activity, liver GPX1 activity, and liver Se concentration in d 18 lactating dams all continued to increase as dietary Se increased with no plateau for these parameters. Reanalysis of those data, however, revealed that the large increases for all markers occurred between 0.025 and 0.05 μg Se/g and that increases between 0.1 and 0.2 μg Se/g were negligible. The important but not apparent result in that study was that liver Se and GPX1 activity levels in pregnant rats were dramatically reduced relative to the nonpregnant rats at every level of dietary Se, thus inferring that additional dietary Se was necessary to meet all of the needs of the dams and pups. The present study shows that downregulation of GPX1 mRNA can explain the declines in GPX1 activity and Se observed in the earlier study (25). The short duration of the preexperiment supplementation period in the previous study is the likely cause of the significant alteration of markers by 0.2 vs. 0.1 μg Se/g diet. Behne et al. (26,27) also reported drops in serum Se and plasma GPX3 activity during pregnancy in rats fed 0.3 μg Se/g diet. In a study using 2nd-generation Se-deficient rat pups supplemented with 0.2 μg Se/g diet, plasma Se and liver Se concentrations in maternal dams were 50 and 65%, respectively, of levels in nonpreg-
nant females (28), further affirming the downregulation of selenoprotein expression during pregnancy and lactation in the rat.

Daily feed intake in this study was not determined. In our previous study evaluating the Se requirement in rapidly growing female rats, weaning rats gained 5.1 g/d over 32 d and weighed 225 g at the end of the study. In the present study, pregnant rats gained 5.9 g/d and thus were growing at approximately the same rate as weaning female rats and weighed ~370 g on d 18 of pregnancy. Similarly, metabolizable energy needs (kJ/BW^{0.75}) of rats in late pregnancy are twice those of nonpregnant rats and can be even higher in late lactation (13). Thus the pregnant and lactating rats in this study were consuming perhaps twice as much diet and thus total Se per day than is consumed by young rapidly growing rats. This additional dietary Se plus the Se released as GPX1 decreases provides the Se needed for fetal growth and for milk production.

This study also provides some tantalizing data at least for rats concerning the role of GPX1 as a Se store or buffer (23,24). Although it is clear that GPX1 can be an important antioxidant enzyme that can protect against acute oxidative challenge or against virus infection, it appears that pregnant rats naturally downregulate the level of expression of GPX1 mRNA, which leads to a decrease in the liver levels of GPX1, thus potentially releasing the Se that was in GPX1 for other metabolic fates or for excretion. Whether this really occurs is not known because Se excretion was not evaluated in these studies, but the observation of Behne et al. (26,27) that serum Se falls 30% in later stages of pregnancy in rats suggests that Se released from the liver is rapidly cleared. Unpublished work in our laboratory showed that the lack of GPX1 in GPX1-knockout mice can impair growth in weanling mice, and this can be readily prevented by as little as 0.02 µg Se/g diet.

In summary, this comprehensive set of studies evaluating Se requirements at d 1, 12, and 18 of pregnancy and d 7 and 18 of lactation clearly shows that dietary Se requirements do not increase during pregnancy and lactation relative to Se requirements in weanling female rats. The dietary Se requirements actually decrease based on some parameters. The Se regulation of liver GPX1 mRNA is unchanged, suggesting that the regulation of this message may be very closely associated with the homeostatic sensor that senses Se and regulates Se status in this metabolically important organ. Second, the unchanged regulation of GPX1 mRNA by dietary Se suggests that this parameter may be a suitable primary marker for assessment of Se status and Se requirements, and that characterization of GPX1 mRNA regulation during the life cycle of rats is necessary to understand the behavior of other, more readily available markers for assessing Se status. Unfortunately, however, it appears that other non-Se factors can radically alter the expression of GPX1. Thus, these studies clearly illustrate that full knowledge of the nature of the regulation of the biomarker used for assessing nutrient status and nutrient requirements must be known before changes in that marker should be attributed to actual changes in Se status or Se requirements. As assessments of Se requirements are expanded from rats to other rodents and to other species including humans, it must be recognized that the differences in the distribution as well as the regulation of these parameters will profoundly affect the apparent Se status. The high levels of GPX1 in rodent liver may have been selected preferentially during evolution to provide a large Se store in this small rodent, but the important Se stores in humans are yet to be determined.

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LITERATURE CITED


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