

**Selenium supplementation of Chinese women with habitually low selenium intake increases plasma selenium, plasma glutathione peroxidase activity, and milk selenium, but not milk glutathione peroxidase activity**

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**Abstract:**

Twenty-one pregnant women living in Xichang County, China, a selenium-deficient area, were divided into two groups and given either a placebo (n = 10) as yeast or selenium-enriched yeast tablets (n = 11) to provide 100 µg selenium per day. This supplementation was begun the last trimester of pregnancy and continued for 3 months after parturition. Plasma selenium levels and glutathione peroxidase (GPX) activity steadily declined in supplemented women, but a curvilinear response occurred in milk selenium and GPX activity in both supplemented and deficient women and in plasma selenium and GPX activity in deficient women. The milk selenium levels were higher in supplemented women but there were no differences in the milk GPX activity between the two groups of women. The plasma ot-tocopherol concentrations declined after parturition in both groups but no differences were found between the two groups of women. Plasma thiobarbituric acid reactive substances declined in supplemented women but showed a curvilinear response in unsupplemented women, suggesting peroxidative stress in these women. GPX, selenium, and peroxidative responses in plasma and milk following parturition is advocated as a new method to assess selenium status of lactating women.

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## Article:

### INTRODUCTION

The quantity of selenium in human milk is influenced by several factors including dietary selenium intake.<sup>1</sup> Milk selenium concentrations in the United States tend to vary from 13 ng/g in areas with low soil selenium such as Ohio to 28 ng/g in areas with high soil selenium such as South Dakota.<sup>2,3</sup> The average human milk selenium concentration in the United States is approximately 16 ng/g.<sup>3-5</sup>

In other areas of the world, human milk selenium concentration can be very different than in the United States. In China, human milk selenium concentrations as low as 7.7 ng/g have been reported in an area with extremely low soil selenium content.<sup>6</sup> In contrast, China also has areas of extremely high soil selenium, such as Enshi, where milk selenium levels reach 94 ng/g and areas of adequate soil selenium, such as Beijing, where milk selenium levels are comparable to those found in the United States (14 ng/g).<sup>6</sup>

Selenium supplementation has been shown to increase milk selenium concentrations. In Illinois, lactating women who were given 200 µg/d of selenium (as selenomethionine) for 4 weeks increased their milk selenium concentration from 15.8 to 19.7 ng/g, a 25% increase.<sup>7</sup> Lactating women in Pennsylvania who were given 20 µg/d selenium as sodium selenate for 3 months experienced a 37.5% increase in milk selenium concentrations, from 24 ng/g milk to 33 ng/g milk.<sup>8</sup>

A strong, positive relationship has been demonstrated between whole blood selenium and milk selenium for residents of New Zealand.<sup>9</sup> However, this same relationship was not seen in residents of Maryland.<sup>10</sup> In Illinois, a positive correlation was seen between plasma selenium and milk selenium.<sup>3</sup> Therefore, although it is not presently known how selenium is transported into human milk,<sup>11</sup> it is thought that a relationship between plasma selenium and milk selenium concentrations exists.

Although plasma selenium concentrations seem to influence milk selenium concentrations, milk is not as sensitive to increased selenium intake as is plasma. When women in New Zealand were supplemented with 50 µg/d of selenium,<sup>12</sup> plasma mean selenium concentration was 67% higher (45 ng/g versus 75 ng/g) in supplemented compared with unsupplemented women. In these same women, mean milk selenium concentration was 12.9 ng/g for women taking the selenium supplement and 9.4 ng/g for those not taking the supplement, an amount that was 37% higher. In a study conducted in China<sup>6</sup> the mean plasma selenium concentration was 380% higher (98 ng/g versus 20.4 ng/g) in women who lived in an area of adequate soil selenium, whereas the mean milk selenium concentration was only 82% higher (14 ng/g versus 7.7 ng/g) in these women compared with the values measured in women from an area of low soil selenium.

Approximately 15 to 30% of the selenium in human milk is associated with glutathione peroxidase (GPX).<sup>13</sup> It is unclear whether GPX in milk functions only as a carrier of selenium or also protects milk from peroxidation.<sup>14</sup> However, a correlation has been shown between milk GPX activity and milk linoleic acid content,<sup>15</sup> suggesting the enzyme may act as an antioxidant. This is consistent with our earlier study showing a higher linoleic acid content in milk from women with an adequate selenium intake versus milk in those with lower selenium status. 6,12

A study of lactating women showed that human milk selenium concentration and GPX activity were correlated, especially when selenium levels were low.<sup>3</sup> Because the habitual dietary intake of selenium of the people who live in the area of China studied in this investigation is extremely low, it is an ideal population for examining such relationships. Therefore, the purpose of this study was to evaluate the effect of selenium supplementation on plasma and milk selenium concentrations and GPX activity over time after parturition in Chinese women with habitually low intakes of dietary selenium. Preliminary reports of this study have been presented previously.<sup>16,17</sup>

## MATERIALS AND METHODS

The study was a single-blind, placebo-controlled, intervention study. Plasma was collected four times and human milk samples were obtained six times from 21 pregnant women between the ages of 20 and 30 years living in Xichang County, China, a rural area in this country with historically low selenium intake. Subject recruitment was conducted by village doctors in collaboration with our Chinese cohort Dr. Yiming Xia of the Chinese Academy of Preventive Medicine in Beijing. The selected women were lifelong residents of several villages in Xichang County. The women had not taken selenium supplements in the year before becoming pregnant, had no known illness, and had been in good health for at least 1 year. The women had similar lifestyles and economic status. Each subject was paid \$50 (U.S.) at the end of the study. The Institutional Review Board of Oregon State University reviewed and approved the study protocol. The study was also approved by a special institutional review board convened at the Chinese Academy of Preventive Medicine in Beijing, China, and oral consent was obtained from each participant.

Eleven of the women recruited for the study were supplemented daily with two selenium-enriched yeast tablets containing a total of 100 µg of selenium, which contained approximately 85% of the selenium as selenomethionine.<sup>18</sup> This selenium source was used because it is the most common one available commercially. The remaining subjects received placebo tablets containing only yeast, which was shown by analysis to contain 0.63 µg selenium per tablet. Both the placebos and the selenium-enriched yeast tablets were supplied by Nutrition 21 (San Diego, CA USA). The subjects began taking their tablets at the beginning of the third trimester of pregnancy and continued until 3 months after the births of their babies. Plasma and human milk selenium concentrations and GPX activities were measured to assess selenium status. *α*-Tocopherol content and lipid peroxidation were measured only in plasma. A fatty acid profile (FAP) was determined in both plasma and milk.

Compliance was monitored by the local doctor and by analysis of plasma and milk selenium concentrations. All subjects completed the study. The data for one subject taking the selenium supplement were removed from the statistical analysis because plasma and milk selenium concentrations were significantly lower than the other subjects in this group, suggesting noncompliance. The Chinese government determined the dietary and nutritional status of the populations<sup>19</sup> living in various regions of the country and this also was used as a basis in our earlier study.<sup>6</sup>

Blood samples were collected within 6 days of births of the babies, and 2, 4, and 12 weeks after parturition. Trained medical personnel collected the blood from the antecubital vein with a sterile needle in an evacuated glass tube containing 0.2 mL of 5% ethylenediamine tetraacetic acid (EDTA) as an anticoagulant. The blood was centrifuged (5 min at 3,000 rpm) and the plasma frozen immediately (-20°C) by members of Dr. Xia's laboratory. At the end of the study period the samples were transported

by air while frozen on dry ice to Oregon State University (OSU). Once at OSU, the plasma was quickly thawed and aliquoted into separate vials and refrozen for future analyses. Samples were stored at -80°C at OSU until they were analyzed. However, the lipid peroxidation assay [thiobarbituric acid reactive substances (TBARS)] was conducted immediately after the first thawing.

Human milk samples were collected on the same days that the plasma samples were collected (0, 2, 4, and 12 weeks after parturition) and also at 1 and 3 weeks afterward. The village doctors instructed the subjects the correct way to clean their breasts with 75% ethanol and express the milk by hand into a sterile, 15 mL container, which was provided. The women expressed approximately 10 mL of foremilk, from one breast, at the beginning of a feeding. The milk sample was aliquoted and frozen (-20°C) for analyses. Human milk samples were transported and stored in the same manner as the plasma samples.

Hematocrit and hemoglobin values were measured in China using a standard clinical procedure. Selenium concentrations in plasma and human milk were determined using a semi-automated

**Table 1** Age of subjects and selected biological values at the time of delivery  
There were no statistically significant differences between the supplemented and unsupplemented groups for any of the variables. Values are means  $\pm$  SEM.

	-Se (n = 10)	+Se (n = 10)
Age (years)	23 $\pm$ 1	25 $\pm$ 1
Hematocrit (%)	41 $\pm$ 2	39 $\pm$ 2
Hemoglobin (g/L)	130 $\pm$ 7	130 $\pm$ 7

fluorimetric method.<sup>20</sup> GPX activity in plasma and human milk was determined as previously described<sup>21</sup> with noted modifications.<sup>22</sup>

Lipid peroxidation was measured by the TBARS method using a fluorescence spectrometer (LS-3B, Perkin-Elmer, San Jose, CA USA).<sup>23</sup>  $\alpha$ -Tocopherol was measured in plasma using a fluorimetric high performance liquid chromatography method.<sup>24,25</sup> The FAP was determined by gas chromatography by a method previously described.<sup>26</sup>

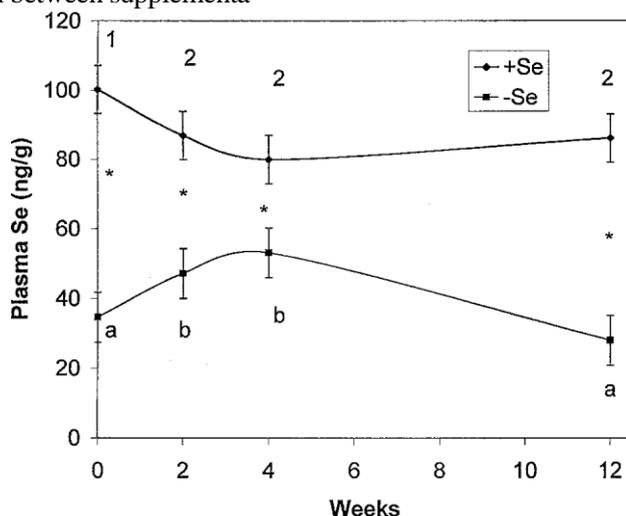
The means and standard errors were calculated for all variables. Data were analyzed using two-way analysis of variance with repeated measures, with the two factors being supplementation and time when the samples were obtained. When there were no interactions between supplementation and time, main effects were evaluated. When interactions between supplementation and time were present, cell means were reported. Correlations between plasma selenium and plasma GPX and between milk selenium and milk GPX were also calculated. No data were transformed. Analysis was performed using SAS 6.11 (SAS Institute Inc., Cary, NC USA). Data were considered statistically significantly different if the *P*-value was less than 0.05.

## RESULTS

The ages of the subjects are given in *Table 1*. There were no statistically significant differences in mean hematocrit and hemoglobin values at the time of birth between the women with or without selenium supplementation (*Table 1*).

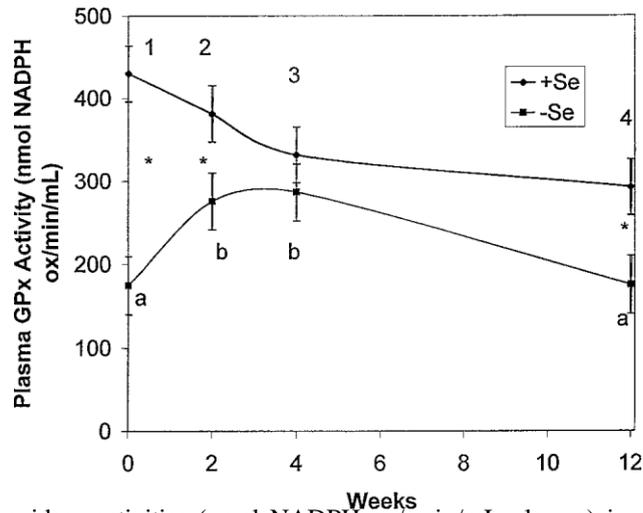
There was an interaction between supplementation and time for plasma selenium concentration. Therefore, cell means are reported. At the time of delivery, the mean plasma selenium concentration of the women who received the supplement was  $100 \pm 7$  ng/g plasma (*Figure 1*). Over the next 2 weeks, the amount of selenium in the plasma dropped to  $85 \pm 7$  ng/g plasma ( $P < 0.0001$ ) and remained constant at  $80 \pm 7$  ng/g and  $86 \pm 7$  ng/g over the next 4 and 12 weeks, respectively. A different pattern, however, was seen in the unsupplemented women (*Figure 1*). The plasma selenium concentration of the unsupplemented women ( $35 \pm 7$  ng/g) was much lower at the time of delivery than that of the supplemented women but rose significantly to  $47 \pm 7$  ng/g ( $P = 0.013$ ) and  $52 \pm 7$  ng/g ( $P = 0.0015$ ), respectively, at 2 and 4 weeks afterwards (*Figure 1*). The plasma selenium then dropped to  $28 \pm 7$  ng/g 12 weeks after delivery, a concentration statistically equivalent to that seen at parturition. Mean plasma selenium concentrations were significantly higher at each time point for the women who received the supplement than for those who did not ( $P < 0.013$ ).

Because there was an interaction between supplementa-

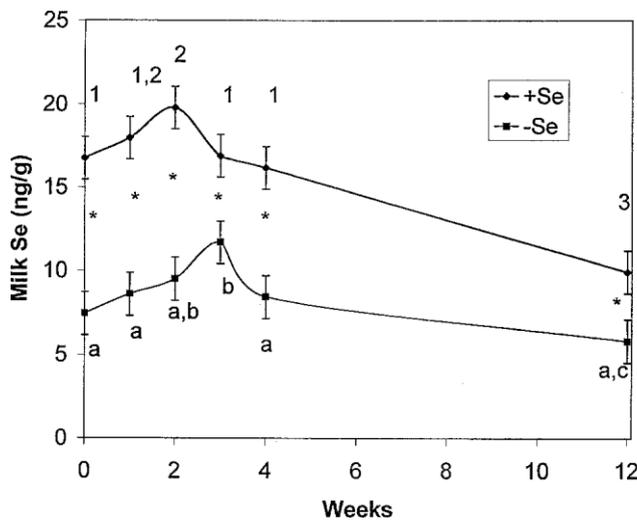


**Figure 1** Plasma selenium concentrations (ng/g plasma) in samples obtained from selenium-supplemented (+Se;  $n = 10$ ) and unsupplemented (—Se;  $n = 10$ ) lactating women in China. Values represent mean  $\pm$  SEM from parturition (0 weeks) to 12 weeks after delivery. Asterisks between lines indicate statistical significance between groups at that time point ( $P < 0.05$ ). Data with different numbers or letters are significantly different within a particular group ( $P < 0.05$ ).

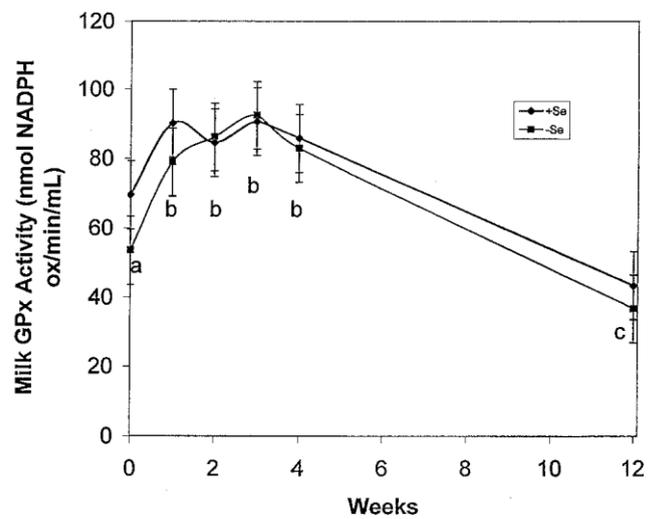
tion and time for plasma GPX activity, cell means are reported. In contrast to the plasma selenium concentrations for the supplemented women, which dropped 2 weeks after parturition and then remained constant, plasma GPX activity was highest at parturition and dropped steadily over time for the supplemented women (*Figure 2*). Two weeks after delivery mean plasma GPX activity was significantly ( $381 \pm 34$  mmol/min/mL) lower than at parturition ( $P =$



**Figure 2** Plasma glutathione peroxidase activities (nmol NADPH ox/ min/mL plasma) in samples obtained from selenium-supplemented (+Se;  $n = 10$ ) and unsupplemented (—Se;  $n = 10$ ) lactating women in China. Values represent mean  $\pm$  SEM from parturition (0 weeks) to 12 weeks after delivery. Asterisks between lines indicate statistical significance between groups at that time point ( $P < 0.05$ ). Data with different numbers or letters are significantly different within a particular group ( $P < 0.05$ ).



**Figure 3** Milk selenium concentrations (ng/g milk) in samples obtained from selenium-supplemented (+Se;  $n = 10$ ) and unsupplemented (—Se;  $n = 10$ ) lactating women in China. Values represent mean  $\pm$  SEM from parturition (0 weeks) to 12 weeks after delivery. Asterisks between lines indicate statistical significance between groups at that time point ( $P < 0.05$ ). Data with different numbers or letters are significantly different within a particular group ( $P < 0.05$ ).



**Figure 4** Milk glutathione peroxidase (GPx) activities (nmol NADPH ox/min/mL) in samples obtained from selenium-supplemented (+Se;  $n = 10$ ) and unsupplemented (—Se;  $n = 10$ ) lactating women in China. Values represent mean  $\pm$  SEM from parturition (0 weeks) to 12 weeks after delivery. Asterisks between lines indicate statistical significance between groups at that time point ( $P < 0.05$ ). Data with different numbers or letters are significantly different within a particular group ( $P < 0.05$ ).

0.014). Four weeks after delivery mean plasma GPX activity was  $332 \pm 34$  mmol/min/mL, a value that was significantly lower than that at 2 weeks ( $P = 0.011$ ). The mean plasma GPX activity for the women who received the supplement was even lower 12 weeks after delivery ( $293 \pm 34$  mmol/min/mL) than 4 weeks after delivery ( $P = 0.044$ ).

Plasma GPX activities for the unsupplemented women followed the same pattern as their plasma selenium concentration (*Figure 2*). The mean GPX activity ( $175 \pm 34$  mmol/min/mL) rose significantly to  $276 \pm 34$  mmol/min/mL 2 weeks after delivery ( $P = 0.0001$ ) and remained at this level ( $277 \pm 36$  mmol/min/mL) 4 weeks after delivery. However, 12 weeks after delivery, mean plasma GPX activity had decreased ( $P = 0.0001$ ) to  $175 \pm 34$  mmol/min/mL, which was statistically equivalent to the mean activity at delivery. Mean plasma GPX activity was significantly higher in women who received the supplement than in the unsupplemented women at all times ( $P < 0.041$ ), except at 4 weeks where it tended to be higher ( $P = 0.09$ ).

There was a significant interaction between supplementation and time for milk selenium concentration (*Figure 3*); hence cell means are discussed. At delivery, the mean milk selenium concentration for the women who received the supplement was  $16.7 \pm 1.3$  ng/g but rose to  $17.9 \pm 1.3$  ng/g and  $19.8 \pm 1.3$  ng/g milk 1 and 2 weeks after delivery, respectively. Even though the mean selenium concentrations were statistically equivalent at delivery and 1 week afterward, the mean selenium concentration 2 weeks after delivery was significantly higher ( $P = 0.035$ ). Mean milk selenium concentrations for these women decreased to  $16.9 \pm 1.3$  ng/g milk and  $16.2 \pm 1.3$  ng/g milk 3 and 4 weeks after delivery, respectively. These values were statistically equivalent to the value at delivery ( $P = 0.044$ ) but significantly lower than the concentration at 2 weeks ( $P = 0.013$ ). Twelve weeks after parturition the mean milk selenium concentration for these women was  $9.9 \pm 1.3$  ng/g milk, the lowest level ( $P = 0.0001$ ) of all time points.

Mean milk selenium concentrations for the unsupplemented women followed a pattern similar to that of the supplemented women (*Figure 3*). The milk selenium ( $7.4 \pm 1.3$  ng/g) at delivery was statistically equivalent to the value measured at 2 weeks. The mean milk selenium concentration rose significantly to  $11.7 \pm 1.3$  ng/g the third week after delivery. This level, although statistically equivalent to the concentration 2 weeks after delivery, was significantly higher than the concentrations seen at delivery and 1 week later ( $P = 0.004$  and  $0.032$ , respectively). The mean milk selenium concentration decreased to  $8.4 \pm 1.3$  ng/g milk 4 weeks after delivery, which was significantly lower than the level 3 weeks after delivery ( $P = 0.024$ ) but statistically equivalent to the values seen at delivery and 1 and 2 weeks afterward. Mean milk selenium was significantly higher at each time point for the women who received the supplement than for the women who did not ( $P = 0.037$ ).

There was no interaction between time and supplementation for milk GPX activity, and thus, although cell means are graphed, main effect means are reported at each time point (*Figure 4*). Mean milk GPX activity increased significantly in both groups of women above the activity seen at parturition 1, 2, 3, and 4 weeks after delivery ( $P < 0.05$ ). Twelve weeks after delivery the mean milk GPX activity for all women was significantly lower than activities at all other points ( $P < 0.05$ ). There were no differences in milk GPX activity at any time between the supplemented and un-supplemented women.

The overall correlations of selenium and GPX were higher in milk and plasma from unsupplemented women than from supplemented women (Table 2). Although still statistically significant, the lowest overall correlation was

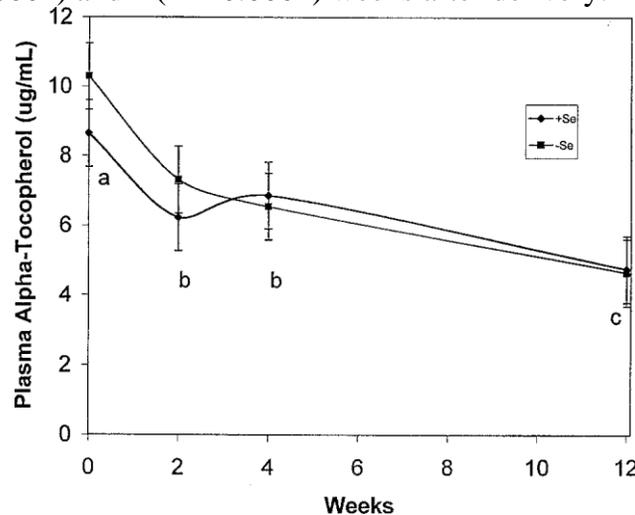
**Table 2** Correlations between selenium and glutathione peroxidase activity in plasma and milk

	<i>r</i>	<i>P</i>
Plasma		
+Se	0.43	0.006
-Se	0.87	0.0001
Milk		
+Se	0.43	0.006
-Se	0.68	0.0001

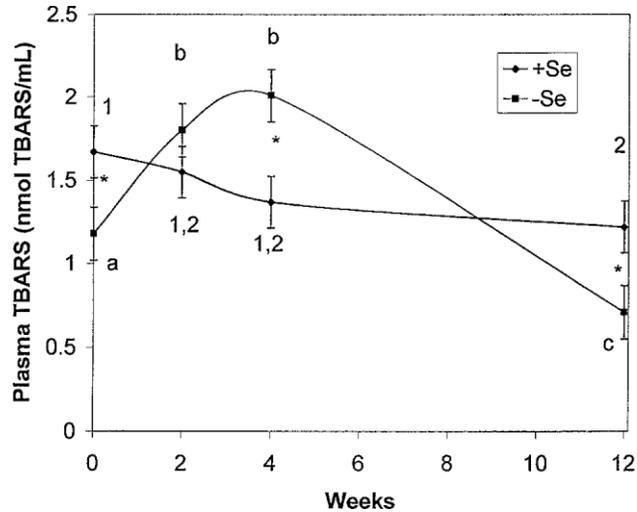
with selenium and GPX activity in plasma of supplemented women.

There was no interaction between time and supplementation for plasma  $\alpha$ -tocopherol and, again, although cell means are graphed, main effect means are reported for each time point (Figure 5). The mean plasma  $\alpha$ -tocopherol concentration was highest at delivery for both groups of women but dropped significantly ( $P < 0.05$ ) 2 and 4 weeks after delivery. A further significant ( $P < 0.05$ ) decrease occurred 12 weeks after delivery. There were no significant differences in plasma  $\alpha$ -tocopherol concentration between the two groups of women at any time.

There was an interaction between supplementation and time for plasma TBARS and therefore cell means are reported (Figure 6). The mean plasma TBARS remained statistically constant 2 and 4 weeks after delivery in the supplemented women. However, 12 weeks after delivery plasma TBARS was significantly lower than levels at delivery ( $P = 0.014$ ). The mean plasma for unsupplemented women showed a completely different pattern. Mean plasma TBARS rose significantly 2 ( $P = 0.0004$ ) and 4 ( $P = 0.0001$ ) weeks after delivery. Twelve weeks after



**Figure 5** Plasma  $\alpha$ -tocopherol concentrations ( $\mu\text{g/mL}$ ) in samples obtained from selenium-supplemented (+Se;  $n = 10$ ) and unsupplemented (-Se;  $n = 10$ ) lactating women in China. Values represent mean  $\pm$  SEM from parturition (0 weeks) to 12 weeks after delivery. Asterisks between lines indicate statistical significance between groups at that time point ( $P < 0.05$ ). Data with different numbers or letters are significantly different within a supplemental group ( $P < 0.05$ ).



**Figure 6** Plasma thiobarbituric acid reactive substances (TBARS) concentrations (nmol TBARS/mL) in samples obtained from selenium- supplemented (+Se;  $n = 10$ ) and unsupplemented (—Se;  $n = 10$ ) lactating women in China. Values represent mean  $\pm$  SEM from parturition (0 weeks) to 12 weeks after delivery. Asterisks between lines indicate statistical significance between groups at that time point ( $P < 0.05$ ). Data with different numbers or letters are significantly different within a particular group ( $P < 0.05$ ).

delivery mean plasma TBARS were significantly lower than at any other time point ( $P = 0.007$ ). Mean plasma TBARS were significantly higher at delivery ( $P = 0.04$ ) and 12 weeks afterward ( $P = 0.038$ ) for women who received the supplement than for those who did not. Two weeks after delivery the mean plasma TBARS values were statistically equivalent between the supplemented and unsupplemented women, whereas women who did not receive the supplement had significantly higher TBARS levels 4 weeks after delivery ( $P = 0.0047$ ). There were no differences in the FAP either in plasma or milk from the two groups of women (data not shown).

## DISCUSSION

The average milk selenium concentration at the initiation of lactation was reported to be approximately 40  $\mu\text{g/L}$  for women with adequate selenium intake.<sup>1</sup> By this standard even the women in the present study who took selenium did not reach adequate levels of selenium in colostrum (approximately 20  $\mu\text{g/L}$ ; *Figure 2*). This suggests that a longer period of supplementation is needed to reach milk selenium levels equal to adequate selenium status. Interestingly, selenium is only one of two elements (the other is iodine) of the major or trace minerals greatly affected by maternal diet.<sup>1</sup>

Plasma selenium concentration (*Figure 1*) and GPX activity (*Figure 2*) in unsupplemented women, milk selenium concentration (*Figure 3*) and GPX activity (*Figure 4*) in both supplemented and unsupplemented women, and TBARS in plasma (*Figure 6*) of unsupplemented women displayed similar patterns after parturition. All five of these variables increased significantly 2 and 4 weeks after parturition and then decreased. This may suggest that the mothers used their selenium reserves to the maximum extent for prevention of lipid peroxidation for protection of their infants. This would appear particularly true for the unsupplemented women where this biological readjustment resulted in peroxidative conditions, as demonstrated by increased TBARS (*Figure 6*). This is supported by our previous data, which indicated no differences in GPX activity in colostrum in women living in deficient versus

adequate selenium areas of China.<sup>6</sup> Interestingly, similar patterns were found for GPX in the brain of selenium-deficient rats.<sup>27</sup> After feeding rats a selenium-deficient diet, the brain GPX activity significantly increased before it returned to initial levels. Based on these data and the present results, we propose that there is a biological readjustment of selenium distribution under limited conditions to meet the requirements of critical tissues such as the brain and colostrum. Further investigations into this protective mechanism would appear to be warranted.

Based on the data in this article, we propose a new method for the evaluation of selenium status of pregnant women. We postulate that the curvilinear responses seen in plasma selenium concentration (*Figure 1*) and GPX activity (*Figure 2*) and in milk selenium concentration (*Figure 3*) and GPX activity (*Figure 4*) are an indication of insufficient selenium and are due to biochemical adjustments to meet the metabolic needs of the infants. The deficient women apparently used the limited body selenium reserves to the maximum extent to produce an optimum amount of GPX, presumably for the benefit of their infants. A steady decline occurred in the plasma selenium levels (*Figure 1*) and GPX activity (*Figure 2*) in the supplemented women, which is consistent with data by other researchers that showed a decline in plasma of women with time after parturition.<sup>7</sup> Using our newly postulated procedure for evaluating the selenium status of pregnant women, the curvilinear response of milk selenium (*Figure 3*) and GPX activity (*Figure 4*) in the supplemented women indicates that they too had not received sufficient selenium to meet the metabolic needs of both themselves and their infants. Even though we assayed only GPX activity, it is possible that other selenoproteins or selenoenzymes could show similar responses as GPX.

The lack of a difference in the FAP supports this hypothesis. There were no differences in the FAP either in plasma or milk between the two groups of women (data not shown). This is in contrast to our earlier data with New Zealand women in whom selenium supplementation was started during the first trimester of pregnancy<sup>12</sup> or with Chinese women living in deficient, adequate, or excessive selenium areas in China.<sup>6</sup> Other researchers have also found a significant correlation between linoleic acid content in milk and GPX activity in human milk.<sup>28</sup> It is suggested that because milk GPX activity was less in both groups, there was not enough GPX activity to protect the linoleic acid from oxidation. Presumably this could be corrected if dietary selenium is increased, such as supplementation for a longer period of time.

The data from the supplemented women (*Figures 1* and *2*) agree with the results by another research group<sup>7</sup> that found decreases in plasma selenium concentration and GPX activity with time after parturition in lactating women of adequate selenium status. Even though most researchers reported a positive correlation of plasma selenium concentration and plasma GPX activity in pregnant women,<sup>3,28</sup> the selenium status is one factor affecting this correlation. A significant positive correlation was found between plasma selenium and plasma GPX activity in pregnant women of low selenium status, but in contrast a negative correlation was found in women of higher selenium status.<sup>29</sup>

Differences in milk selenium levels (*Figure 3*) but not in GPX activities (*Figure 4*) are consistent with our prior work with New Zealand women.<sup>12</sup> Researchers do not agree on whether milk selenium is correlated with milk GPX activity. Consistent with our previous data there was no correlation between milk selenium and milk GPX activity in Polish women,<sup>30</sup> but other

investigators have reported correlations between milk selenium and milk GPX from women in Japan,<sup>31</sup> Africa<sup>32</sup> (early milk), and the United States (Illinois).<sup>3</sup> In addition to selenium, other factors obviously can affect GPX activity in milk. For example, stage of lactation is a factor because a correlation of milk selenium and GPX activity was found in early lactation but not late lactation in African women.<sup>32</sup> In work with vegetarian versus nonvegetarian women, the levels of selenium content and GPX activity in milk of vegetarians could not be explained by dietary selenium intakes only.<sup>15</sup> Even though milk selenium concentrations are more than four times higher in women living in Enshi, China, than in those living in Beijing, there were no differences in milk GPX activity between these two groups of women,<sup>6</sup> further suggesting that other factors in addition to selenium effect milk GPX activity.

The higher correlations of selenium with GPX in both plasma and milk (*Table 2*) indicate a greater amount of selenium with GPX in the unsupplemented women than in the supplemented ones. This is consistent with other data showing greater amounts of selenium with other selenoproteins with increased selenium intakes.<sup>29</sup>  $\alpha$ -Tocopherol in the plasma was not affected by the selenium supplement but a decline was found with time after parturition (*Figure 5*). This decline in plasma  $\alpha$ -tocopherol after parturition is consistent with other research reports.<sup>33,34</sup> Despite  $\alpha$ -tocopherol concentration being the same in both groups, the TBARS were different, suggesting that other factors influenced this response.

Plasma TBARS for the supplemented women remained fairly constant over the entire course of the 12 weeks, decreasing toward the end (*Figure 6*). However, plasma TBARS for the unsupplemented women rose significantly 2 and 4 weeks after delivery and then fell, suggesting peroxidative stress in the unsupplemented women during lactation. By 12 weeks after delivery, plasma TBARS in the unsupplemented women were again lower than those in the supplemented women, further suggesting biochemical readjustments with limited selenium reserves. It is realized that TBARS lack specificity and sensitivity, and more acceptable methods will be used in future studies.

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