Selenium, selenoproteins and selenometabolites in mother and baby at time of birth.

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ABSTRACT

Deficiency of selenium (Se), an essential micronutrient, has been implicated in adverse pregnancy outcomes. Our study was designed to determine total serum Se, selenoproteins [extracellular glutathione peroxidase (GPx-3), selenoprotein P (SeP)], selenoalbumin (SeAlb) and selenometabolites (SeMetab) in healthy women and their newborns at delivery. A cross-sectional study included 83 healthy mother-baby couples. Total Se and Se-species concentrations were measured in maternal and umbilical-cord serum by an in-series coupling of two-dimensional size-exclusion and affinity high-performance liquid chromatography (2D/SE-AF-HPLC). Additional measurements were made of serum SeP concentration by ELISA and of serum GPx-3 enzyme activity. Total Se concentration was significantly higher in maternal serum than in cord serum (68.9±15.2 and 56.1±14.6 µg/L respectively; \(p<0.01\)). There were significant correlations between selenoprotein and SeAlb concentrations in mothers and newborns, although they also showed significant differences: GPx-3 (11.2±3.7 vs 10.5±3.5 µg/L; \(p<0.01\)) SeP (42.5±9.5 vs 28.1±7.7 µg/L; \(p<0.01\)) and SeAlb (11.6±3.6 vs 14.1±4.3 µg/L; \(p<0.01\)) in maternal and cord serum, respectively. Serum GPx-3 activity and concentration were correlated positively in mothers (\(r=0.33; p=0.038\)) but not in newborns. GPx-3 activity in cord serum was significantly correlated with gestational age (\(r=0.44; p=0.009\)). SeAlb concentration was significantly higher in babies while SeP and GPx-3 were significantly higher in mothers. The differences cannot be explained by simple diffusion; specific transfer mechanisms are probably involved. GPx-3 concentrations in mothers at delivery are related to maternal Se status, while the GPx-3 activity in cord serum depends on gestational age.
INTRODUCTION

Selenium (Se) is an essential micronutrient with a wide range of protective functions, which are exerted through its incorporation into selenoproteins (1). Inadequate dietary Se intake can compromise human reproduction since Se deficiency has been associated with obstetric and perinatal complications such as male and female infertility, miscarriage, pre-eclampsia, intrauterine growth restriction, preterm delivery and neural defects in the offspring (2-4).

The maternal-fetal transfer mechanisms for Se and selenoproteins have been studied in mice (5) and in placental models in vitro (6), leading to the identification of some specific placental transporters (6,7). Though a number of studies have measured Se concentrations in mothers and newborns at the time of birth, they have had contradictory results (8-10). To some extent, these contradictions might be explained by differences in Se status between geographic regions (11), distribution patterns between Se species and the reliability of the method of determination of Se (12). Whatever the case, those studies were limited by measurement only of total serum/plasma Se which does not allow distinction between different species of Se that may be transferred from mother to baby by different mechanisms.

In investigating the relative concentrations of Se in mother and newborn it is important to take account of the different Se components in serum/plasma (13): two selenoproteins [selenoprotein P1 (SeP) (14) and the extracellular glutathione peroxidase GPx-3], Se incorporated non-specifically as selenomethionine (SeMet) in lieu of methionine in albumin (SeAlb) and other plasma proteins, and a small amount of non-protein-bound low molecular weight Se metabolites (SeMetab). SeP is the most abundant selenoprotein in plasma and is a good indicator of Se status in non-Se-replete humans (15). The activity of GPx-3 in human serum is a complementary marker of Se status. Importantly, GPx-3 has been identified as a key enzyme in the defence against oxidative stress during decidualization (the post-ovulatory process of endometrial remodeling in preparation for implantation) by reducing H$_2$O$_2$ in the endometrium (16).

We have been able to determine these species in serum from mother and baby pairs at time of birth owing to our previous development of a two-dimensional chromatographic method for simultaneous speciation of serum GPx-3, SeP, SeAlb and SeMetab (17). Although the accuracy of this method has been assessed by analyzing a commercial human serum (BCR-637), it has not previously been applied in a clinical setting.

The aims of the current study were: (i) to determine simultaneously Se, selenoproteins, SeAlb and SeMetab concentrations by this new method in both maternal and cord sera from uncomplicated pregnancies at the time of birth; (ii) to compare maternal and neonatal concentrations in order to
elucidate potential maternal-fetal transfer mechanisms; (iii) to assess whether SeP measured by 2D/SE-AF-HPLC corresponded to that measured by ELISA and to assess the coherence between GPx-3 measured both as a concentration and an enzyme activity. Our study may also help to assess Se requirements in pregnancy, as recommended by the European Food Safety Authority (EFSA) Panel (18).

METHODS

A cross-sectional study was performed on 83 healthy mother-baby couples. Participants were recruited at random from pregnant women without any maternal or neonatal risk factors, who gave birth at term, at the Department of Obstetrics and Gynecology, Hospital de Riotinto, during the years 2014-2015. Women with diseases, multiple gestation and perinatal complications such as obstructed labor, low Apgar score (below 5) or where there was suspicion of infant pathology, were excluded.

A full history was taken from the enrolled mothers which included whether or not they took a multivitamin/mineral supplement [a standard formula approved by the Department of Health, containing 200 µg iodide (I-), 55µg Se, as sodium selenite, 10 mg zinc and 200 mg docosahexaenoic acid (DHA)] during pregnancy. Medical data from clinical examination of mothers and babies were also recorded at birth.

This study was approved by the local Ethics Committee and written informed consent was obtained from all the participants. Since we also collected samples from cord blood, the parents provided separate, signed, informed consent for this specific purpose.

Analytical procedures

Blood samples were collected from mothers during the 24 hours before delivery. At birth, a sample of cord blood was obtained and its pH was measured. Serum was separated and frozen at -80°C until further analysis.

Se and selenoprotein concentrations were measured by a two-dimensional chromatographic method that used in-series size-exclusion, and high performance affinity liquid chromatography (2D/SE-AF-HPLC), as previously described; this method overcomes common spectral interferences from chloride and bromide (17). Briefly, plasma and serum samples were filtered before injecting on to the chromatographic platform, which connected two in-series, stacked, 5 ml HiTrap®Desalting columns (for size exclusion separation), and a dual-affinity column arrangement comprising a 1 ml heparin-sepharose column (HEP-HP, able to retain selectively SeP) and a 1 ml blue-sepharose column (BLU-HP that retains both SeP and SeAlb) interconnected by a 6-port column-switching valve, in series
coupled to the ICP-MS system by means of a 30 cm PEEK tubing (0.6 mm i.d.) for Se detection. Ammonium acetate 0.05 M (at pH 7.5), Phase A, and 1.5 M Phase B, were successively used as mobile phase. Post-column isotope dilution analysis was performed by the introduction of $^{74}$Se via a T connector.

Chromatographic performance was checked regularly by measuring control standards to ensure enough separation between species and method sensitivity after a considerable number of analyses. The proposed speciation method has been validated using a CRM of human serum (BCR-637) certified for total Se content ($\text{SeTotal}=81 \pm 7 \text{ ng mL}^{-1}$). The concentration of different Se species in BCR-637 obtained with this method has been compared with previous results for this CRM from Jitaru et al. The relative standard deviation (RSD, %) and the detection limits (LD, ng g$^{-1}$ of Se) follow: GPx-3, RSD 21 and DL 0.2; SeP, RSD 12 and DL 0.7; SeAlb, RSD 9 and DL 0.9.

SeP concentration was also analyzed by enzyme immunoassay (ELISA) (USCN Business Co., Ltd., Houston, TX), and GPx-3 activity by a commercial kit (Cayman Chemical, Ann Arbor, MI).

Statistical analysis

Sample size calculation: With 38 subjects per mother-baby group, and a standard deviation of 15 µg/L of Se in mean serum Se concentration of 71 µg/L [obtained from a recent study of Spanish pregnant women (71.2± 14.9)](20), our study had 80% power to detect a difference of at least 10 µg/L in total serum Se between mother and baby groups at a two-sided, 5% significance level. We included 83 healthy mother-baby couples.

As our sample included women who took a multivitamin/mineral supplement and women who did not, a comparison of means between these two groups was made to see whether this supplementation had a significant effect. Assuming a two-sided hypothesis, the conventional alpha and beta levels, and a proportion of 3:1 for the studied groups (the use of multivitamin/mineral supplements in our population barely reached a quarter of pregnant women), the sample size calculated was 19 women for the supplemented group and 57 pregnant women for the non-supplemented group. We included 21 and 62 women, respectively.

Data are presented as medians and standard deviations for continuous variables and as percentages for categorical variables. The contrast hypothesis for two samples was evaluated using Fisher’s exact test for categorized variables and Student’s t-test for continuous variables. Wilcoxon signed-rank test was used to compare matched samples. The correlation between variables was determined using the Spearman test, designing multiple linear regression models in those cases where it was desired to predict the variance adjusted for other variables, besides the main variable. The contrast hypothesis
for more than two samples was determined with an analysis of variance (ANOVA). All P-values were
two-sided, and statistical significance was declared at $P < 0.05$. All data were analyzed using SPSS
20.0 (IBM SPSS Statistics).

RESULTS

Maternal and neonatal characteristics

Table 1 shows maternal and neonatal characteristics. Since exclusively healthy pregnant women were
recruited, the cesarean section rate was only 7.5% (compared to the usual cesarean section rate at our
center of around 16%), and perinatal outcomes were good (there were no admissions to the Neonatal
Intensive Care Unit).

No correlations were found between maternal age, parity, body mass index or level of education and
maternal Se concentration, nor were any correlations found between neonatal weight at birth or
gestational age with maternal or neonatal serum Se concentrations. However, there was a negative
correlation between hours of fasting prior to blood draw and total Se concentration in maternal serum
($r = -0.30; p<0.05$).

Women who did not take multivitamin /mineral supplements (containing 55 µg Se) were significantly
younger than women who did take them (29.42±5.35 vs 31.16±4.80 years, respectively; $p<0.05$). No
significant differences in parity, body mass index or level of education were found between women
who took multivitamin/mineral supplements and those who did not (data not shown).

Se and selenoprotein concentrations by 2D/SE-AF-HPLC

Total Se and selenoprotein concentrations in maternal and cord serum are summarized in Table 2.
Total Se, GPx-3 and SeP concentrations were significantly higher in maternal than in cord serum,
while SeAlb was significantly more concentrated in cord serum. There was no significant difference
in the SeMetab concentration between maternal and cord serum. The chromatograms of maternal and
umbilical cord sera are shown in Figure 1.

The correlations between maternal and cord Se species were significant in all cases, reaching the
highest value for GPx-3 and the lowest for SeP.

The proportion of Se species in maternal and cord serum was different; while SeP accounted for 65%
of total Se in maternal serum, it only represented 50% of total Se in cord serum. By contrast, SeAlb
constituted 15% of total Se in maternal serum but 28% in cord serum. The percentages for GPx-3 and
SeMetab were similar in maternal and cord sera: 14% for GPx-3 in both, and 7% and 8% for SeMetab
in maternal and cord sera, respectively (Figure 2a). When the Se species concentrations are represented graphically, the different pattern in maternal and cord serum is apparent (Figure 2b).

There were no significant differences in the concentrations of Se species in maternal or cord serum between women who took multivitamins/minerals containing Se and those who did not (Table 3).

Maternal total serum Se was strongly correlated with all the Se species in maternal serum, i.e. GPx-3 ($r=0.71; p<0.01$), SeP ($r=0.89; p<0.01$), SeAlb ($r=0.80; p<0.01$) and SeMetab ($r=0.42; p<0.01$), and moderately correlated with the concentrations of the Se species in cord serum, i.e. total Se in cord serum ($r=0.56; p<0.01$), GPx-3 ($r=0.47; p<0.01$), SeP ($r=0.43; p<0.01$), SeAlb ($r=0.45; p<0.01$) and SeMetab ($r=0.48; p<0.01$).

Total Se in cord serum was correlated with all the Se species studied in cord serum, i.e. GPx-3 ($r=0.78; p<0.01$), SeP ($r=0.89; p<0.01$), SeAlb ($r=0.80; p<0.01$) and SeMetab ($r=0.60; p<0.01$), and moderately correlated with the concentration of the Se species in maternal serum, i.e. GPx-3 ($r=0.64; p<0.01$), SeP ($r=0.32; p<0.01$), SeAlb ($r=0.61; p<0.01$) and SeMetab ($r=0.66; p<0.01$).

Negative correlations were found between umbilical-cord pH and maternal SeAlb ($r=-0.33; p=0.01$), cord total Se ($r=-0.30; p=0.04$) and cord SeAlb ($r=-0.30; p=0.02$).

**GPx-3 activity**

GPx-3 activity was significantly higher in maternal than in cord serum (112.87±68.91 and 38.67±25.03 nmol/min/ml, respectively; $p<0.01$), and there was no correlation between the activities ($r=0.12; p=0.48$). There was a significant correlation between GPx-3 concentration and its activity in maternal serum ($r=0.33; p=0.04$), but this correlation was not apparent in cord serum (data not shown). Maternal GPx-3 activity also correlated significantly with total Se concentration in maternal serum ($r=0.36; p=0.03$) and with SeMetab concentration in cord serum ($r=0.50; p<0.01$), but not with other Se species in either maternal or cord serum (data not shown). GPx-3 activity in cord serum was significantly correlated with birth weight ($r=0.39; p=0.02$) and gestational age ($r=0.43; p<0.01$). This last correlation remained significant after adjusting by birth weight and height ($R^2=0.23; p=0.04$). However, GPx-3 activity in cord serum did not correlate with total Se or Se species either in cord or maternal serum (data not shown).

**SeP measured by ELISA.**

SeP concentration determined by ELISA was significantly different in maternal and cord serum (38.27±12.34 µg/L and 1.93±0.98 µg/L respectively, $p<0.01$). No correlation was found between SeP in maternal and cord serum when measured by ELISA. There was no correlation between SeP
concentrations measured by HPLC and ELISA in either maternal or cord serum (data not shown).

Although SeP concentration in maternal serum measured by HPLC and ELISA were not significantly different (42.49 ± 9.49 µg/L and 38.27 ± 12.34 µg/L respectively, $p=0.10$), there was a significant difference between these two methods of determination in cord serum (28.06 ± 7.69 µg/L by HPLC and 1.93 ± 0.98 µg/L by ELISA, $p<0.01$). Maternal SeP measured by ELISA correlated significantly with GPx3 concentration in cord serum ($r=0.33; p=0.04$).

**DISCUSSION**

This is the first study to analyse simultaneously total Se and Se species in maternal and cord serum at the time of birth using a new optimized HPLC method. Although some of these factors have previously been measured at delivery, the interrelationships between the different Se species in maternal and cord sera are largely unknown, as is the biological significance of such relationships. Our findings indicate that, at birth, total Se, GPx-3 and SeP concentrations are significantly higher in maternal than in cord serum while the opposite is the case for SeAlb. In general, simple diffusion cannot explain the maternal-neonatal transfer of Se and Se species around the time of birth.

Oxidative stress is tightly related to perinatal morbidity and mortality, so the antioxidant selenoproteins may have a central role in protecting against adverse pregnancy outcomes$^{(21)}$. Thus total Se concentration and GPx-3 activity in cord serum were found to increase with advancing pregnancy$^{(22)}$ while oxidative stress during labor was associated with elevated fetal GPx-3 activity$^{(23)}$. A study in rats showed that a sufficient Se supply was required to ensure antioxidative protection to the fetus during the oxygen transformations that take place during delivery and early postnatal life$^{(24)}$. By contrast, in a UK population of slightly higher Se status than ours, maternal blood Se concentrations fell from 12 to 35 weeks of gestation (believed to be partly due to the expansion of plasma volume and partly due to receptor-mediated transfer of SeP from mother to fetus) and maternal GPx-3 activity did not change$^{(25)}$.

Our study found lower Se concentrations than those reported in other studies of Spanish pregnant women$^{(20,26)}$. Using the most restrictive cut-off of 70 µg/L of total serum Se concentration required to optimize GPx-3 activity$^{(27)}$, 57 of 83 (69%) pregnant women in our study had insufficient serum Se to fulfill this requirement. However, based on alternative estimates of the range of serum Se concentration required for optimal GPx-3 activity, i.e., 89-114 µg/L$^{(11)}$, 90% of the women in our sample did not reach this optimal status.
The dietary intake of Se is the major determinant factor of serum-Se concentration. Since we used data from medical records, information on dietary Se intake was not available. However, other factors have been reported to be associated with Se status such as sex (28,29), age, BMI (28), education (30), socioeconomic status (28,30) or physical activity (29), as well as location (31). In this regard, our patients came from a non-urban area in southern Spain where Se intake is traditionally low (31). Furthermore, most of them were overweight or obese, with a low level of education, had never worked or were unemployed. All these factors lead us to speculate that they were unlikely to eat the traditional diet with high consumption of vegetables and cereals (32).

It might have been expected that pregnant women who took multivitamins/multiminerals containing sodium selenite would have had significantly higher concentrations of at least some Se species than those who did not, as sodium selenite can be used for the synthesis of selenoproteins such as GPx-3 or SeP (though it will not increase SeAlb) (12). However, the absence of higher concentrations in the women on supplements is not altogether surprising as the Se dose was relatively low (55 µg/day), only 25% of the women took such supplements and, we have no data on their compliance. Furthermore, in vivo human studies showed that selenite is only moderately absorbed (between 56-85%) (33).

In recent years, Se status in maternal and cord blood at delivery has been determined in a number of studies, though with varied outcomes (34-36). Al-Saleh et al. (34) examined the role of Se in reducing oxidative stress induced by cadmium and its impact on birth measures. They concluded that the extent of benefit afforded by Se is not governed only by its concentration but also by the different chemical forms of Se that interact with various proteins. Chen et al. (35) attempted to clarify concentrations for cadmium, mercury, lead and Se in mothers and newborns and their placental transfer. Finding a high degree of maternal-fetal Se transfer, they concluded that there was free trans-placental passage of Se from mother to fetus. However, our results do not support that conclusion; the different pattern of transfer of SeP and SeAlb implies that different mechanisms are involved in the transfer of these different Se species from mother to fetus.

It appears likely that receptor-mediated uptake exists in humans as previously found in pregnant mice (5). Burk and colleagues identified two mechanisms of Se transfer; from early to mid-gestation, plasma GPx-3 and SeP were transported via the uterine fluid, probably by pinocytosis, whereas in the latter half of gestation, placental transfer of maternal SeP occurred through the apoE receptor 2 (apoER2) (5). In fact, the higher levels of SeAlb in cord serum compared to maternal serum suggest the existence of a further maternal-fetal Se transfer mechanism (5,37). Studies in mice and rats suggest that this other pathway involves the transfer of Se as SeMet-containing proteins or SeMet itself (via
methionine transporters) to the fetus\(^5,37\). This mechanism depends on the amount of SeMet in the diet of the mother, appears to be unregulated and is highly dependent on the mother’s Se status, thus it will be less effective under conditions of Se deficiency\(^5\). The similar concentrations for SeMetabol and their strong correlation in maternal and cord serum can be explained by a simple diffusion mechanism of trans-placental passage that does not occur for other Se species.

GPx-3 activity was measured to see whether there was a correlation between GPx-3 concentrations and GPx-3 activity. As expected, the GPx-3 activity in maternal serum significantly correlated with maternal total Se and GPx-3 concentrations, but GPx-3 activity in cord serum only correlated with gestational age and birth weight. These results are in agreement with previous findings\(^38\); it seems that GPx-3 activity in mothers at delivery is related to maternal Se status, while the GPx-3 activity in cord serum depends on gestational age\(^22\).

Finally, our study compared different methods of determining SeP: 2D/SE-AF-HPLC and ELISA. The growing replacement of traditional immunoassay techniques by liquid chromatography-tandem mass spectrometry (LC-MS/MS) has highlighted the advantages and limitations of each system as well as the disparity in results by the two methods\(^39\). These two methods have been previously used and published\(^17,40\). In our sample, the concentration of SeP in maternal serum measured by both methods gave a similar result, but this was not the case in cord serum where SeP concentration measured by ELISA was significantly lower than that measured by HPLC, and did not correlate with any other parameters. While these results may suggest that ELISA is not a reliable method of quantifying SeP in cord serum, other explanations are possible. SeP has three transcripts in humans although all variants begin with the same non-coding exon\(^41\). The most abundant transcript in all tissues was SePa. However, all variants (SePa, b and c) showed tissue and developmental stage-specific expression patterns in neonatal and adult tissues\(^41\). These transcripts can synthetize different isoforms, varying in size and Se content\(^41\) as previously proposed\(^42\). Moreover, the silencing of each transcript results in a differential synthesis of SeP isoforms\(^41\). In this respect, the antibodies used in ELISA kits could have a different affinity for the SeP isoforms present in serum. This could affect the binding of the antibody to the SeP protein in the ELISA and therefore give different results than techniques that do not use antibodies. A further determination of SeP levels by the use of kits from other suppliers could be considered but unfortunately we have no remaining cord blood samples. Alternatively, this discordance in the results of SeP concentration between 2D/SE-AF-HPLC and ELISA may suggest that in the newborn, the predominant SeP isoform is different from that found in the mothers, and perhaps, with different functions\(^41\). It is also likely that full-length SeP has a higher Se content that the shorter isoforms\(^15\). It has been suggested that while liver exports full-length SeP, other tissues secrete mostly shorter SeP isoforms, possibly for local use as a redox enzyme or as a
signaling molecule\textsuperscript{(15)}. Our finding supports the hypothesis that circulating truncated SeP isoforms might not reach the concentrations necessary for successful identification by ELISA\textsuperscript{(43)}. The current study has some limitations. Firstly, the absence of a food frequency questionnaire did not allow us to assess Se intake. Furthermore, the small proportion (25\%) of women taking a supplement containing Se and the lack of compliance data may have prevented our finding significantly higher markers of status in that group.

Our study has also certain strengths; our design made possible the collection of data and samples from healthy pregnant women and their newborns at the time of birth. The multiple biomarkers studied from the same patients and the availability of a robust and reliable method to determine simultaneously different Se species gave us an insight into the relationships between these Se species in mother and newborn.

**CONCLUSION**

There is a different pattern of Se species in maternal and cord serum at the time of birth which suggests the existence of different mechanisms of trans-placental passage of Se species from mother to fetus that are species-dependent.

The lack of concordance in the SeP concentration measured by 2D/SE-AF-HPLC and ELISA suggests the possibility that the predominant SeP isoform in newborn may be different from that found in the mother; this possibility needs to be followed up in a later study.

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**Conflict of Interest:**
The authors declared that no competing interests exist.

**Authorship:**

*Santos and Velasco contributed equally to this study, designing the study, recruiting participants, collecting the samples and writing the manuscript. García-Fuentes performed the biochemical tests and the statistical analysis. García-Barrera and Gómez-Ariza designed the analytical experiments, the instrumental couplings and supervised the results. Santos and Callejón-Leblic performed the analytical experiments and the tandem mass spectrometry analysis. Rayman contributed intellectually to the study and to the writing of the manuscript.*
REFERENCES


31. Matos-Reyes MN, Cervera ML, Campos RC, et al. (2010) Total content of As, Sb, Se, Te and Bi in Spanish vegetables, cereals and pulses and estimation of the contribution of these foods to the Mediterranean daily intake of trace elements. Food Chem 122, 188-94.


**Table 1:** Main characteristics of participants.

<table>
<thead>
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<th>Maternal characteristics</th>
<th>Mean</th>
<th>SD</th>
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<td></td>
</tr>
<tr>
<td>Gestational age at birth (days)</td>
<td>275.2</td>
<td>8.4</td>
</tr>
<tr>
<td>Birth weight (gr)</td>
<td>3392.84</td>
<td>408.91</td>
</tr>
<tr>
<td>Birth height (cm)</td>
<td>50.32</td>
<td>1.56</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>33.48</td>
<td>1.44</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>33.40</td>
<td>1.67</td>
</tr>
</tbody>
</table>
Table 2: Comparisons between concentrations of total Se, selenoproteins, SeAlb and SeMetab in maternal and cord sera measured by 3D/SE-AF-AEC-HPLC.

<table>
<thead>
<tr>
<th></th>
<th>Maternal serum</th>
<th>Cord serum</th>
<th>Correlation (r; p)</th>
<th>Difference in means (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Se (µg/L)</td>
<td>68.95 ± 15.23</td>
<td>56.14 ± 14.61</td>
<td>0.56 &lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GPx-3 concentration (µg/L)</td>
<td>11.24 ± 3.68</td>
<td>10.46 ± 3.48</td>
<td>0.74 &lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SeP concentration (µg/L)</td>
<td>42.49 ± 9.49</td>
<td>28.06 ± 7.69</td>
<td>0.25 &lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SeAlb (µg/L)</td>
<td>11.35 ± 3.59</td>
<td>14.14 ± 4.26</td>
<td>0.60 &lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SeMetab (µg/L)</td>
<td>4.32 ± 2.86</td>
<td>3.98 ± 2.38</td>
<td>0.60 &lt;0.01</td>
<td>n.s</td>
</tr>
</tbody>
</table>
Table 3: Concentration of various Se species in maternal and cord serum in women who took multivitamin/mineral supplement containing Se during pregnancy and those who did not take such supplements.

<table>
<thead>
<tr>
<th></th>
<th>No supplements (N=62)</th>
<th>Multivitamins (N=21)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Total Se (µg/L)</td>
<td>67.40 ± 14.24</td>
<td>69.72 ± 15.32</td>
<td>0.54</td>
</tr>
<tr>
<td>Maternal GPx-3 (µg/L)</td>
<td>10.84 ± 3.38</td>
<td>11.57 ± 3.92</td>
<td>0.43</td>
</tr>
<tr>
<td>Maternal SeP (µg/L)</td>
<td>41.44 ± 9.04</td>
<td>44.86 ± 10.25</td>
<td>0.16</td>
</tr>
<tr>
<td>Maternal SeAlb (µg/L)</td>
<td>11.16 ± 3.38</td>
<td>11.09 ± 3.31</td>
<td>0.41</td>
</tr>
<tr>
<td>Maternal SeMetab (µg/L)</td>
<td>4.35 ± 2.96</td>
<td>3.21 ± 1.45</td>
<td>0.18</td>
</tr>
<tr>
<td>Cord Total Se (µg/L)</td>
<td>55.24 ± 13.32</td>
<td>55.12 ± 11.75</td>
<td>0.97</td>
</tr>
<tr>
<td>Cord GPx-3 (µg/L)</td>
<td>10.13 ± 3.24</td>
<td>10.84 ± 3.52</td>
<td>0.42</td>
</tr>
<tr>
<td>Cord SeP (µg/L)</td>
<td>28.12 ± 7.70</td>
<td>27.12 ± 6.36</td>
<td>0.62</td>
</tr>
<tr>
<td>Cord SeAlb (µg/L)</td>
<td>13.49 ± 3.46</td>
<td>14.51 ± 3.62</td>
<td>0.28</td>
</tr>
<tr>
<td>Cord SeMetab (µg/L)</td>
<td>3.82 ± 2.16</td>
<td>3.67 ± 2.26</td>
<td>0.83</td>
</tr>
</tbody>
</table>
Figure 1: Selenoprotein chromatograms in maternal and cord sera.
Figure 2: Comparison between (a) percentage of selenoproteins and selenometabolites in maternal and cord sera (b) concentrations of total selenium, selenoproteins and selenometabolites in maternal and cord sera.